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PTO/SB/05 (2/98)

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**UTILITY  
PATENT APPLICATION  
TRANSMITTAL**

Only for new nonprovisional applications under 37 CFR 1.53(b)

Attorney Docket No. 05686.0004.NPUS00/35-10(17108)  
First Named Inventor or Application Identifier Karunanandaa  
Title Nucleic Acid Molecules and Other Molecules Associated  
Sterol Synthesis and Metabolism  
Express Mail Label No.

**APPLICATION ELEMENTS**

See MPEP chapter 600 concerning utility patent application contents

Assistant Commissioner for Patents  
**ADDRESS TO:** Box Patent Application  
Washington, DC 20231

1. ☐ \*Fee Transmittal Form (Form PTO-1082)  
(Submit an original and a duplicate for fee processing)
2. ☒ Specification [Total Pages 159]  
(preferred arrangement set forth below)  
- Descriptive title of the Invention  
- Cross References to Related Applications  
- Statement Regarding Fed sponsored R&D  
- Reference to Microfiche Appendix  
- Background of the Invention  
- Brief Summary of the Invention  
- Brief Description of the Drawings (if filed)  
- Detailed Description  
- Claims  
- Abstract of the Disclosure
3. ☐ Drawing(s) (35 USC 113) [Total Sheets ]
4. ☐ Oath or Declaration [Total Pages ]  
a. ☐ Newly executed (original or copy)  
b. ☐ Copy from a prior application (37 CFR 1.63(d))  
(for continuation/divisional with Box 17 completed)  
[Note Box 5 below]  
i. ☐ **DELETION OF INVENTOR(S)**  
Signed statement attached deleting inventor(s) named  
in the prior application, see 37 CFR 1.63(d)(2) and  
1.33(b).
5. ☐ Incorporation By Reference (useable if Box 4b is checked)  
The entire disclosure of the prior application, from which a copy  
of the oath or declaration is supplied under Box 4b, is considered  
as being part of the disclosure of the accompanying application  
and is hereby incorporated by reference therein.

6. ☐ Microfiche Computer Program (Appendix)
7. Nucleotide and/or Amino Acid Sequence Submission  
(if applicable, all necessary)  
a. ☒ Computer Readable Copy  
b. ☒ Paper Copy (identical to computer copy)  
c. ☒ Statement verifying identity of above  
copies

**ACCOMPANYING APPLICATION PARTS**

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Return Receipt Postcard (MPEP 503) (Two)  
(should be specifically itemized)
14. ☐ \*Small Entity Statement(s) ☐ Statement filed in prior application, Status still proper and desired
15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
16. ☐ Other:

\*NOTE FOR ITEMS 1 & 14 IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28)

**17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:**

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: /

Prior Application Information: Examiner: Group/Art Unit:

**18. CORRESPONDENCE ADDRESS**

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(Insert Customer No. or Attach bar code label here)

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Signature				Date	July 11, 2000

Burden Hour Statement. This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231 DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.



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July 11, 2000

Assistant Commissioner for Patents  
Washington, D.C. 20231

Box Patent Application

Re: U.S. Non-Provisional Utility Patent Application  
Application No.: To Be Assigned  
Filed: Herewith  
For: **Nucleic Acid Molecules and Other Molecules  
Associated with Sterol Synthesis and Metabolism**  
Inventors: Balasulojini KARUNANANDAA *et al.*  
Atty. Docket: 05686.0004.NPUS00/35-21(17108)

Sir:

The following documents are forwarded herewith for appropriate action by the U.S.  
Patent and Trademark Office:

1. Utility Patent Application Transmittal (PTO/SB/05);
2. U.S. Utility Patent Application entitled:  
**Nucleic Acid Molecules and Other Molecules Associated with  
Sterol Synthesis and Metabolism**  
and naming as inventors:  
**Balasulojini KARUNANANDAA, Jaehyuk YU, and Ganesh M. KISHORE,**  
the application consisting of:
  - a. A specification containing:
    - (i) 154 pages of a description prior to the claims;
    - (ii) 3 page of claims (27 claims);
    - (iii) a one (1) page abstract; and
    - (iv) 777 pages of a sequence listing;
3. A computer-readable diskette containing the sequence listing;
4. Statement Regarding Sequence Submission; and



5. Two (2) return postcards.

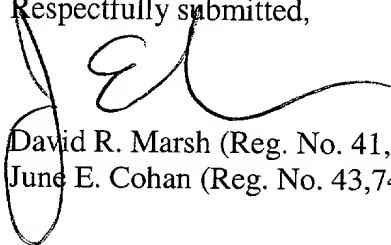
This application claims priority under 35 U.S.C §119(e) of U.S. Provisional Application No. 60/142,981, filed July 12, 1999.

This application is being filed without an executed Declaration, and without payment of official fees.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the sequence listing and the computer readable copy of the sequence listing submitted herewith in the above application are the same.

It is respectfully requested that, of the two attached postcards, one be stamped with the filing date of these documents and returned to our courier, and the other, prepaid postcard, be stamped with the filing date and unofficial application number and returned as soon as possible.

Respectfully submitted,

  
David R. Marsh (Reg. No. 41,408)  
June E. Cohan (Reg. No. 43,741)

Enclosures

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

1c808 U.S. PTO  
09/614221  
07/11/00

In re application of:

Balasulojini KARUNANANDAA *et al.*

Art Unit: To Be Assigned

Appln. No.: To Be Assigned

Examiner: To Be Assigned

Filed: July 11, 2000

Atty. Docket: 05686.0004.NPUS00/  
35-21(17108)

For: Nucleic Acid Molecules and Other  
Molecules Associated with Sterol  
Synthesis and Metabolism

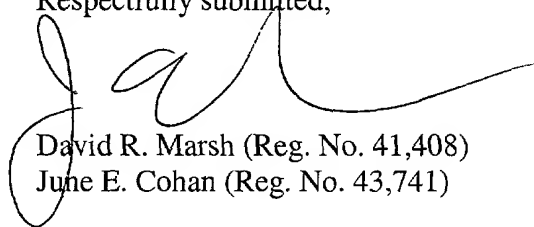
**Statement Regarding Sequence Submission**

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above-mentioned application are the same.

Respectfully submitted,

  
David R. Marsh (Reg. No. 41,408)  
June E. Cohan (Reg. No. 43,741)

Date: July 11, 2000

Howrey Simon Arnold & White, LLP  
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# NUCLEIC ACID MOLECULES AND OTHER MOLECULES ASSOCIATED WITH STEROL SYNTHESIS AND METABOLISM

## FIELD OF THE INVENTION

This invention relates to the field of biotechnology, particularly as it pertains to the  
5 production of sterols in a variety of host systems particularly plants. More specifically, the  
invention relates to nucleic acid molecules encoding proteins and fragments of proteins  
associated with sterol and phytosterol metabolism as well as the encoded proteins and fragments  
of proteins and antibodies capable of binding to them. The invention also relates to methods of  
using the nucleic acid molecules, fragments of the nucleic acid molecules, proteins, and  
10 fragments of proteins. The invention also relates to cells, organisms, particularly plants, or seeds,  
or progeny of plants, that have been manipulated to contain increased levels or overexpress at  
least one sterol or phytosterol compound.

## BACKGROUND OF THE INVENTION

Sterols are a class of essential, natural compounds required by all eukaryotes to complete  
15 their life cycle. The types of sterols produced and predominantly present within each of the  
phylogenetic kingdoms varies. Plants produce a class of sterols called phytosterols. A  
phytosterol called sitosterol predominates. In animals, cholesterol is typically the major sterol  
while in fungi it is ergosterol.

Phytosterols from plants possess a wide spectrum of biological activities in animals and  
20 humans. Phytosterols are considered efficacious cholesterol-lowering agents (Pelletier *et al.*,  
*Annals Nutrit. Metab.* 39:291-295 (1995), the entirety of which is herein incorporated by  
reference). Lower cholesterol levels are linked to a reduction in the risk to cardiovascular  
disease. Phytosterols can also block cholesterol absorption in the intestine, which would also  
lead to lower cholesterol levels. Thus, enhancing the levels of phytosterols in edible plants and  
25 seeds, or products derived from these plants and seeds, may lead to food products with increased  
nutritive or therapeutic value.

In one aspect, this invention provides these desirable plants and seeds as well as methods to produce them. Since, as will be discussed below, the genetic manipulation made possible by this invention involves families of related genes that cross phylogenetic boundaries, the effects are not limited to plants alone.

## 5 **Biochemistry of Sterol Synthesis**

A number of the important sterol biosynthetic enzymes, reactions, and intermediates have been described. Sterol synthesis uses acetyl CoA as the basic carbon building block. Multiple acetyl CoA molecules form the five-carbon isoprene units, hence the name isoprenoid pathway. Enzymatic combination of isoprene units leads to the thirty-carbon squalene molecule, which is  
10 the penultimate precursor to sterols.

Throughout plants, animals, and fungus, the reactions proceed as: acetyl CoA →  
HMGCoA, mevalonate, mevalonate 5 phosphate, mevalonate 5-pyrophosphate, isopentyl  
diphosphate, 5-pyrophosphatemevalonate, isopentyl pyrophosphate (PIP), dimethylallyl  
pyrophosphate (DMAPP), PIP + DMAPP, geranyl pyrophosphate + IPP, farnesyl pyrophosphate,  
15 2 farnesyl pyrophosphate, squalene and squalene epoxide

From squalene epoxide, the sterol biosynthesis pathway of plants diverges from that of animals and fungi. In plants, cycloartenol is produced next by cyclization of squalene epoxide. The plant pathway eventually leads to the synthesis of the predominant phytosterol, sitosterol.

Animals go on to produce lanosterol from squalene epoxide, eventually leading to  
20 cholesterol, which is the precursor to steroid hormones and bile acids, among other compounds. In fungi, lanosterol leads to the production of the predominant sterol, ergosterol.

An important regulatory control step within the pathway consists of the HMGCoA →  
Mevalonate step, catalyzed by HMGCoA reductase, and the condensation of 2 farnesyl  
pyrophosphates → squalene, catalyzed by squalene synthase. An early, reported rate-limiting  
25 step, in the pathway is the HMGCoA reductase-catalyzed reaction.

A number of studies have focused on the regulation of HMGCoA reductase. HMGCoA reductase (EC 1.1.1.34) catalyzes the reductive conversion of HMGCoA to mevalonic acid

(MVA). This reaction is a reported controlling step in isoprenoid biosynthesis. The enzyme is regulated by feedback mechanisms and by a system of activation kinases and phosphatases (Gray, *Adv. Bot. Res.*, 14: 25 (1987); Bach *et al.*, *Lipids*, 26: 637 (1991); Stermer *et al.*, *J. Lipid Res.*, 35: 1133 (1994), all of which are herein incorporated by reference in their entirety).

5 Another important regulation occurs at the squalene synthase step. Squalene synthase (EC 2.5.1.21) reductively condenses two molecules of FPP in the presence of  $Mg^{2+}$  and NADPH to form squalene. The reaction involves a head-to-head condensation and forms a stable intermediate, presqualene diphosphate. The enzyme is subject to regulation similar to that of HMGCoA reductase and acts by balancing the incorporation of FPP into sterols and other  
10 compounds.

The sterol pathway of plants diverges from that in animals and fungi after squalene epoxide. In plants, the cyclization of squalene epoxide occurs next, under the regulated control of cycloartenol synthase (EC 5.4.99.8). The cyclization mechanism proceeds from the epoxy end into a chair-boat-chair-boat sequence that is mediated by a transient C-20 carbocationic  
15 intermediate. The reported rate-limiting step in plant sterol synthesis occurs in the next step, S-adenosyl-L-methionine:sterol C-24 methyl transferase (EC 2.1.1.41) ( $SMT_I$ ) catalyzing the transfer of a methyl group from a cofactor, S-adenosyl-L-methionine, to the C-24 center of the sterol side chain. This is the first of two methyl transfer reactions. The second methyl transfer reaction occurs further down in the pathway and has been reported to be catalyzed by  $SMT_{II}$ . An  
20 isoform enzyme,  $SMT_{II}$ , catalyzes the conversion of 24-methylene lophenol to 24-ethylidene lophenol (Fonteneau *et al.*, *Plant Sci Lett* 10:147-155(1977), the entirety of which is herein incorporated by reference). The presence of two distinct SMTs in plants were further confirmed by cloning cDNAs code the enzymes from *Arabidopsis* (Husselstein *et al.*, *FEBS Lett* 381:87-92(1996), the entirety of which is herein incorporated by reference), soybean (Shi *et al.*, *J Biol Chem* 271: 9384-9389(1996), the entirety of which is herein incorporated by reference), maize  
25 (Grebenok *et al.*, *Plant Mol Biol* 34: 891-896(1997), the entirety of which is herein incorporated by reference) and tobacco (Bouvier-Nave *et al.*, *Eur J Biochem* 246: 518-529 (1997); Bouvier-

Nave *et al.*, *Eur J Biochem* 256: 88-96(1998), both of which are herein incorporated by reference in their entirety).

Later in the pathway, a sterol C-14 demethylase catalyzes the demethylation at C-14, removing the methyl group and creating a double bond. Interestingly, this enzyme also occurs in plants and fungi, but at a different point in the pathway. Sterol C14-demethylation is mediated by a cytochrome P-450 complex. A large family of enzymes utilize the cytochrome P-450 complex. There is, in addition, a family of cytochrome P450 complexes. For example, sterol C-22 desaturase (EC 2.7.3.9) catalyzes the formation of the double bond at C-22 on the side chain. The C-22 desaturase in yeast, which is the final step in the biosynthesis of ergosterol, contains a cytochrome P450 that is distinct from the cytochrome P450 participating in the demethylation reaction. Additional cytochrome P450 enzymes participate in brassinosteroid synthesis (Bishop, *Plant Cell* 8:959-969 (1996), the entirety of which is herein incorporated by reference).

Brassinosteroids are steroidal compounds with plant growth regulatory properties, including modulation of cell expansion and photomorphogenesis (Arteca, *Plant Hormones, Physiology, Biochemistry and Molecular Biology* ed. Davies, Kluwer Academic Publishers, Dordrecht, 66 (1995), Yakota, *Trends in Plant Science* 2:137-143 (1997), both of which are herein incorporated by reference in their entirety).

One class of proteins, oxysterol-binding proteins, have been reported in humans and yeast (Jiang *et al.*, *Yeast* 10: 341-353 (1994), the entirety of which is herein incorporated by reference).

These proteins have been reported to modulate ergosterol levels in yeast (Jiang *et al.*, *Yeast* 10: 341-353 (1994)). In particular, Jiang *et al.*, reported three genes KES1, HES1 and OSH1, which encode proteins containing an oxysterol-binding region.

#### **Enzyme Inhibitors and Modulators**

Self-regulatory and feedback regulatory mechanisms of some of the sterol synthesis enzymes provide opportunities to effect sterol metabolism. For example, the introduction of the feedback inhibitor molecule inhibits enzyme action while the removal of that molecule up-regulates the enzyme. In certain circumstances, non-wild type enzymes can effect normal

regulation. These organisms can be generated, for example, by traditional genetic crosses, mutation treatments and through molecular genetics. One example is the overexpression of plant HMGC<sub>o</sub>A reductase in transgenic plants resulting in a 6-10 fold increase in the total sterol levels (for example, transgenic tobacco plants overproducing phytosterols in Schaller *et al.*, *Plant*  
5 *Physiol.* 109: 761 (1995), the entirety of which is herein incorporated by reference).

A number of compounds have been identified that, at least partially, exert their effects on sterol synthesis. For example, mevinolinic acid and lovastatin are competitive inhibitors of HMGC<sub>o</sub>A reductase and zaragonic acid is a competitive inhibitor of squalene synthase (Alberts *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 77:3957-61 (1993); Bergstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 90:80-84 (1980), both of which are herein incorporated by reference). Many fungicides  
10 and insecticides act by inhibiting enzymes, such as those noted above or the C-14 demethylase enzyme (*Sterol Biosynthesis Inhibitors and Anti-feeding Compounds*, Kato *et al.*, Springer-Verlag, New York (1986); *Sterol biosynthesis inhibitors: pharmaceutical and agrochemical aspects*, eds. Berg and Plempel, Ellis Horwood, Chichester, England (1988), both of which are  
15 herein incorporated by reference in this entirety).

However, the use of these compounds can have toxic effects that preclude their use in products destined for animal or human consumption. Furthermore, the increase or decrease in sterol levels possible using these compounds is limited. Typically, the changes in levels occur over a wide spectrum of the pathway. New and more effective methods for manipulating sterol  
20 synthesis are desired.

The present invention provides a gene, *Hes1*, involved in plant phytosterol production. Expression of HES1 (protein) in organisms such as plants can increase phytosterol biosynthesis. The present invention also provides transgenic organisms expressing a HES1 protein, which can enhance food and feed sources.

## 25 SUMMARY OF THE INVENTION

The present invention includes a substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 622.

The present invention includes a substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 1 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 622.

5 The present invention includes a substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 623.

The present invention includes a substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 2 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 623.

The present invention includes a substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 624.

The present invention includes a substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 3 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 624.

The present invention includes a substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 625.

The present invention includes a substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 4 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 625.

The present invention includes a substantially purified nucleic acid molecule comprising a nucleic acid sequence which encodes a plant HES1 protein.

25 The present invention includes an antibody capable of specifically binding a protein comprising the amino acid sequence of SEQ ID NO: 622.



The present invention includes an antibody capable of specifically binding a protein comprising the amino acid sequence of SEQ ID NO: 623.

The present invention includes an antibody capable of specifically binding a protein comprising the amino acid sequence of SEQ ID NO: 624.

5 The present invention includes an antibody capable of specifically binding a protein comprising the amino acid sequence of SEQ ID NO: 625.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to (B) a structural nucleic acid molecule,  
10 wherein the structural nucleic acid molecule comprises a nucleic acid sequence encoding a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragment thereof; which is linked to (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

15 The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to (B) a transcribed nucleic acid molecule with a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the  
20 group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or fragment thereof; which is linked to (C) a 3' non-translated sequence that functions in plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention also provides a method for determining a level or pattern in a plant of a protein in a plant comprising: (A) incubating, under conditions permitting nucleic acid  
25 hybridization, a marker nucleic acid molecule, the marker nucleic acid molecule selected from the group of marker nucleic acid molecules which specifically hybridize to a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 1 through SEQ ID NO: 621 or

complements thereof, with a complementary nucleic acid molecule obtained from the plant cell or plant tissue, wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant permits the detection of an mRNA for the enzyme; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue; and (C) detecting the level or pattern of the complementary nucleic acid, wherein the detection of the complementary nucleic acid is predictive of the level or pattern of the protein in the plant.

The present invention also provides a method for determining a level or pattern of a protein in a plant under evaluation which comprises assaying the concentration of a molecule, whose concentration is dependent upon the expression of a gene, the gene specifically hybridizes to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof, in comparison to the concentration of that molecule present in a reference plant with a known level or pattern of the protein, wherein the assayed concentration of the molecule is compared to the assayed concentration of the molecule in the reference plant with the known level or pattern of the protein.

The present invention also provides a method for determining a mutation in a plant whose presence is predictive of a mutation affecting a level or pattern of a protein comprising the steps: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid, the marker nucleic acid selected from the group of marker nucleic acid molecules which specifically hybridize to a nucleic acid molecule having a nucleic acid sequence selected from the group of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof and a complementary nucleic acid molecule obtained from the plant, wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant permits the detection of a polymorphism whose presence is predictive of a mutation affecting the level or pattern of the protein in the plant; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the

plant; and (C) detecting the presence of the polymorphism, wherein the detection of the polymorphism is predictive of the mutation.

The present invention also provides a method of producing a plant containing an overexpressed protein comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region has a nucleic acid sequence selected from group consisting of SEQ ID NO: 1 through SEQ ID NO: 621, wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in overexpression of the protein; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing an overexpressed protein comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region encodes a protein comprising an amino acid sequence selected from group consisting of SEQ ID NO: 622 through SEQ ID NO: 626, wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in overexpression of the protein; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing reduced levels of a protein comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621; wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of

polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in co-suppression of the protein; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing reduced levels of a protein comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region encodes a protein comprising an amino acid sequence selected from group consisting of SEQ ID NO: 622 through SEQ ID NO: 626; wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in co-suppression of the protein; and (B) growing the transformed plant.

The present invention also provides a method for reducing expression of a protein in a plant comprising: (A) transforming the plant with a nucleic acid molecule, the nucleic acid molecule having an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or fragments thereof and the transcribed strand is complementary to an endogenous mRNA molecule; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and (B) growing the transformed plant.

The present invention also provides a method for reducing expression of a protein in a plant comprising: (A) transforming the plant with a nucleic acid molecule, the nucleic acid molecule having an exogenous promoter region which functions in a plant cell to cause the

production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid encodes a protein comprising an amino acid sequence selected from group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof and the transcribed strand is complementary to an endogenous mRNA molecule; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and (B) growing the transformed plant.

The present invention also provides a method of determining an association between a polymorphism and a plant trait comprising: (A) hybridizing a nucleic acid molecule specific for the polymorphism to genetic material of a plant, wherein the nucleic acid molecule has a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragment of either; and (B) calculating the degree of association between the polymorphism and the plant trait.

The present invention also provides a method of isolating a nucleic acid that encodes a protein or fragment thereof comprising: (A) incubating under conditions permitting nucleic acid hybridization, a first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragment of either with a complementary second nucleic acid molecule obtained from a plant; (B) permitting hybridization between the first nucleic acid molecule and the second nucleic acid molecule obtained from the plant; and (C) isolating the second nucleic acid molecule.

The present invention also provides a method for producing a protein or fragment thereof in an organism comprising introducing a vector comprising a nucleic acid of the present invention and expressing the protein or fragment.

## DETAILED DESCRIPTION OF THE INVENTION

One skilled in the art can refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include *Current Protocols in Molecular Biology* Ausubel, *et al.*, eds., John Wiley & Sons, N.Y. (1989), and supplements through September (1998), *Molecular Cloning, A Laboratory Manual* (Sambrook *et al.*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), for example, each of which are specifically incorporated by reference in their entirety). These texts can also be referred to in making or using an aspect of the invention.

The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response.

The term "substantially purified", as used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native state. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native state.

The agents of the invention may also be recombinant. As used herein, the term recombinant means any agent (*e.g.*, DNA, peptide etc.), that is, or results, however indirect, from human manipulation of a nucleic acid molecule.

It is understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent (*e.g.*, fluorescent labels, Prober *et al.*, *Science* 238:336-340 (1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448, all of which

are hereby incorporated by reference in their entirety). It is further understood that the invention provides recombinant bacterial, mammalian, microbial, archaebacterial, insect, fungal, and plant cells as well as viral constructs comprising the agents of the invention.

**(a) Nucleic Acid Molecules**

5 Agents of the invention include nucleic acid molecules and, more preferably, nucleic acid molecules of maize, soybean, canola, yeast, or *Arabidopsis*. In addition, a number of different plants can be the ultimate source of the nucleic acid molecules of the invention. An exemplary group of genotypes includes: B73 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.); B73 x Mo17 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.); DK604 (Dekalb Genetics,  
10 Dekalb, Illinois U.S.A.); H99 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.); RX601 (Asgrow Seed Company, Des Moines, Iowa); and Mo17 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.). And an exemplary group of soybean types includes: Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa); C1944 (United States Department of Agriculture (USDA) Soybean Germplasm Collection, Urbana, Illinois U.S.A.); Cristalina (USDA Soybean  
15 Germplasm Collection, Urbana, Illinois U.S.A.); FT108 (Monsoy, Brazil); Hartwig (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.); BW211S Null (Tohoku University, Morioka, Japan), PI507354 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.); Asgrow A4922 (Asgrow Seed Company, Des Moines, Iowa U.S.A.); PI227687 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.); PI229358 (USDA Soybean Germplasm  
20 Collection, Urbana, Illinois U.S.A.); and Asgrow A3237 (Asgrow Seed Company, Des Moines, Iowa U.S.A.).

A particularly preferred embodiment of the nucleic acid molecules of the present invention are plant nucleic molecules that comprise a nucleic acid sequence which encodes an oxysterol-binding protein consensus sequence, for example, soybean HES1 (SEQ ID NOS: 622,  
25 623 and 624), and maize HES1 (SEQ ID NO: 625).

Another particularly preferred embodiment of the nucleic acid molecules of the present invention are yeast nucleic acid molecules that comprise a nucleic acid sequence which encodes an oxysterol-binding protein consensus sequence, for example yeast HES1 (SEQ ID NO: 626).

A particularly preferred embodiment of the nucleic acid molecules of the invention are nucleic acid molecules that encode a protein or fragment thereof where the protein or fragment thereof is selected from the group consisting of a HES1, HMGC<sub>o</sub>A reductase, squalene synthase, cycloartenol synthase, SMTI, SMTII and UPC2. In a more particularly preferred embodiment of the nucleic acid molecules of the present invention are nucleic acid molecules that encode a protein or fragment thereof where the protein or fragment thereof is selected from the group consisting of a fungal, more preferably a yeast HES1, a plant, more preferably a maize, soybean or *Arabidopsis* HES1, a plant, more preferably a rubber or an *Arabidopsis* HMGC<sub>o</sub>A reductase, a plant, more preferably an *Arabidopsis* squalene synthase, a plant, more preferably an *Arabidopsis* cycloartenol synthase, a plant, more preferably an *Arabidopsis* SMTI or SMTII and a fungus, more preferably a yeast UPC2.

In a preferred embodiment, the nucleic molecule encodes a HES1 protein, preferably a plant HES1 protein comprising an oxysterol-binding protein consensus sequence -- E(K, Q) xSH (H, R) PPx (S, T, A, C, F)A. In a further preferred embodiment, the nucleic acid molecule encodes a HES1 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In a further preferred embodiment, the nucleic acid molecule molecules encodes a HES1 protein with a conservative amino acid substitution in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In a further preferred embodiment, the nucleic acid molecule molecules encodes a HES1 protein with between 2 and 5 conservative amino acid substitutions in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In a further preferred embodiment, the nucleic acid molecule molecules encodes a HES1 protein with between 5 and 10 conservative amino acid substitutions in an amino acid sequence



selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and  
SEQ ID NO: 625. In a further preferred embodiment, the nucleic acid molecule encodes a HES1  
protein with more than 10 conservative amino acid substitutions in an amino acid sequence  
selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and  
5 SEQ ID NO: 625.

In another preferred embodiment, the nucleic acid molecule encodes a HES1 protein,  
preferably a yeast HES1 protein comprising an oxysterol-binding protein consensus sequence --  
E(K, Q) xSH (H, R) PPx (S, T, A, C, F)A. In a further preferred embodiment, the nucleic acid  
molecule encodes a HES1 protein comprising an amino acid sequence SEQ ID NO: 626. In a  
10 further preferred embodiment, the nucleic acid molecule molecules encodes a HES1 protein with  
a conservative amino acid substitution in amino acid sequence SEQ ID NO: 626. In a further  
preferred embodiment, the nucleic acid molecule molecules encodes a HES1 protein with  
between 2 and 5 conservative amino acid substitutions in an amino acid sequence SEQ ID NO:  
626. In a further preferred embodiment, the nucleic acid molecule molecules encodes a HES1  
15 protein with between 5 and 10 conservative amino acid substitutions in an amino acid sequence  
SEQ ID NO: 626. In a further preferred embodiment, the nucleic acid molecule encodes a HES1  
protein with more than 10 conservative amino acid substitutions in an amino acid sequence SEQ  
ID NO: 626.

In an aspect of the present invention, one or more of the nucleic acid molecules of the  
20 present invention differ in nucleic acid sequence from those encoding a protein or fragment  
thereof in SEQ ID NO: 1 through SEQ ID NO: 621 due to the degeneracy in the genetic code in  
that they encode the same protein but differ in nucleic acid sequence. In another further aspect of  
the present invention, one or more of the nucleic acid molecules of the present invention differ in  
nucleic acid sequence from those encoding a protein or fragment thereof in SEQ ID NO: 1  
25 through SEQ ID NO: 621 due to fact that the different nucleic acid sequence encodes a protein  
having one or more conservative amino acid residue. Examples of conservative substitutions are

set forth in Table 1. It is understood that codons capable of coding for such conservative substitutions are known in the art.

**Table 1**

5	<u>Original Residue</u>	<u>Conservative Substitutions</u>
	Ala	Ser
	Arg	Lys
	Asn	Gln; His
	Asp	Glu
10	Cys	Ser; Ala
	Gln	Asn
	Glu	Asp
	Gly	Pro
	His	Asn; Gln
15	Ile	Leu; Val
	Leu	Ile; Val
	Lys	Arg; Gln; Glu
	Met	Leu; Ile
	Phe	Met; Leu; Tyr
20	Ser	Thr
	Thr	Ser
	Trp	Tyr
	Tyr	Trp; Phe
	Val	Ile; Leu

25 In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those encoding a protein or fragment thereof set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or fragment thereof due to the fact

that one or more codons encoding an amino acid has been substituted for a codon that encodes a nonessential substitution of the amino acid originally encoded.

One subset of the nucleic acid molecules of the invention is fragment nucleic acids molecules. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, the nucleic acid molecules of the invention, such as those specifically disclosed. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 400 nucleotide residues and more preferably, about 15 to about 30 nucleotide residues, or about 50 to about 100 nucleotide residues, or about 100 to about 200 nucleotide residues, or about 200 to about 400 nucleotide residues, or about 275 to about 350 nucleotide residues).

A fragment of one or more of the nucleic acid molecules of the invention may be a probe and specifically a PCR probe. A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure with another nucleic acid. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 ([www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi)), STSPipeline ([www-genome.wi.mit.edu/cgi-bin/www-STSPipeline](http://www-genome.wi.mit.edu/cgi-bin/www-STSPipeline)), or GeneUp (Pesole *et al.*, *BioTechniques* 25:112-123 (1998)), for example, can be used to identify potential PCR primers.

As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the “complement” of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit “complete complementarity” when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be “minimally complementary” if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional “low-stringency” conditions. Similarly, the molecules are said to be “complementary” if they can hybridize to one another with sufficient stability to permit

them to remain annealed to one another under conventional "high-stringency" conditions.

Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989) and by Haymes *et al.*, *Nucleic Acid Hybridization, A Practical Approach*, IRL Press,

5 Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

10 Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 X SSC at 20-25°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of about 0.2 X SSC at 65°C. In addition, the temperature in the wash step can be increased from  
15 low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the invention will specifically hybridize to  
20 one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or more preferably to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO: 29 or complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

25 In a particularly preferred embodiment, a nucleic acid of the invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or more preferably

to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO: 29 or complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

In one aspect of the invention, the nucleic acid molecules of the invention have one or  
5 more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or  
complements thereof or fragment thereof or more preferably to a nucleic acid molecule having  
SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO: 29 or complements  
thereof. In another aspect of the invention, one or more of the nucleic acid molecules of the  
invention share between about 100% and 70% sequence identity with one or more of the nucleic  
10 acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or  
more preferably to a nucleic acid molecule having a nucleic acid sequence selected from the  
group consisting of SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO:  
29 or complements thereof. In a further aspect of the invention, one or more of the nucleic acid  
molecules of the invention share between about 100% and 90% sequence identity with one or  
15 more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or  
complements thereof or more preferably to a nucleic acid molecule having a nucleic acid  
sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID  
NO: 6 through SEQ ID NO: 29 or complements thereof. In a more preferred aspect of the  
invention, one or more of the nucleic acid molecules of the invention share between about 100%  
20 and 95% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID  
NO: 1 through SEQ ID NO: 621 or complements thereof or more preferably to a nucleic acid  
molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1  
through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO: 29 or complements thereof. In an  
even more preferred aspect of the invention, one or more of the nucleic acid molecules of the  
25 invention share between about 100% and 99% sequence identity with one or more of the  
sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or more  
preferably to a nucleic acid molecule having a nucleic acid sequence selected from the group

consisting of SEQ ID NO: 1 through SEQ ID NO: 4, SEQ ID NO: 6 through SEQ ID NO: 29, or complements thereof.

In a preferred embodiment the percent identity calculations are performed using the Megalign program of the LASERGENE bioinformatics computing suite (default parameters,  
5 DNASTAR Inc., Madison, Wisconsin).

In a preferred embodiment of the present invention, the nucleic acid molecule of the present invention encodes a protein or fragment thereof, where a protein exhibits a BLAST probability score of greater than 1E-12, preferably a BLAST probability score of between about 1E-30 and about 1E-12, even more preferably a BLAST probability score of greater than 1E-30  
10 with its homologue.

In a preferred embodiment of the present invention, the nucleic molecule of the present invention encodes a protein or fragment thereof where a protein exhibits a BLAST score of greater than 120, preferably a BLAST score of between about 1450 and about 120, even more preferably a BLAST score of greater than 1450 with its homologue.

15 Nucleic acid molecules of the present invention can comprise sequences that encode a protein or fragment thereof. Such proteins or fragments thereof include homologues of known proteins in other organisms.

A nucleic acid molecule of the invention can also encode a homolog protein. As used herein, a homolog protein molecule or fragment thereof is a counterpart protein molecule or  
20 fragment thereof in a second species (*e.g.*, maize HES1 is a homolog of *Arabidopsis* HES1). A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original protein (*see*, for example, U.S. Patent 5,811,238).

Particularly preferred homologues are selected from the group consisting of alfalfa,  
25 *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, maize, peanut, pepper, potato, rice, rye, sorghum, soybean, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce,

lentils, grape, banana, tea, turf grasses, sunflower, soybean, and *Phaseolus*. A particularly preferred group of homologues are crops harvested for seed oils, including but not limited to rapeseed (high erucic acid rape and canola), maize, soybean, safflower, sunflower, cotton, peanut, flax, oil palm and *Cuphea*.

5 In a preferred embodiment, nucleic acid molecules having SEQ ID NO: 1 through SEQ ID NO: 621 or complements and fragments of either can be utilized to obtain such homologues.

The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006, the entirety of which is herein incorporated by reference).

10 Agents of the invention include nucleic acid molecules that encode a substantially purified nucleic acid molecules encoding at least about a 10 amino acid region, more preferably a 20, 30, 40, or 50 amino acid region, of a protein selected from the group consisting of a fungal, more preferably a yeast HES1, a plant, more preferably a maize, soybean or *Arabidopsis* HES1, a plant, more preferably a rubber or an *Arabidopsis* HMGC<sub>o</sub>A reductase, a plant, more preferably an *Arabidopsis* squalene synthase, a plant, more preferably an *Arabidopsis* cycloartenol synthase, a plant, more preferably an *Arabidopsis* SMTI or SMTII and a fungus, more preferably a yeast UPC2.

#### **(b) Protein and Peptide Molecules**

20 A class of agents comprises one or more of the protein or fragments thereof or peptide molecules having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or one or more of the protein or fragment thereof and peptide molecules encoded by other nucleic acid agents of the invention. A particular preferred class of proteins are those having an amino acid sequence selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 625 or fragments thereof.

25 As used herein, the term “protein molecule” or “peptide molecule” includes any molecule that comprises five or more amino acids. It is well known in the art that proteins may undergo modification, including post-translational modifications, such as, but not limited to, disulfide

bond formation, glycosylation, phosphorylation, or oligomerization. Thus, as used herein, the term "protein molecule" or "peptide molecule" includes any protein molecule that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer to all naturally occurring L-amino acids. This definition is meant to include norleucine, norvaline,  
5 ornithine, homocysteine, and homoserine.

One or more of the protein or fragment of peptide molecules may be produced via chemical synthesis, or more preferably, by expressing in a suitable bacterial or eukaryotic host. Suitable methods for expression are described by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York*  
10 (1989), or similar texts.

A "protein fragment" is a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein. A protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein is a "fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as  
15 keyhole limpet hemocyanin, etc.). Fusion protein or peptide molecules of the invention are preferably produced via recombinant means.

Another class of agents comprise protein or peptide molecules or fragments or fusions thereof comprising SEQ ID NO: 622 through SEQ NO: 625 or fragment thereof or encoded by SEQ ID NO: 1 through SEQ ID NO: 621 in which conservative, non-essential or non-relevant  
20 amino acid residues have been added, replaced or deleted. Computerized means for designing modifications in protein structure are known in the art (Dahiyat and Mayo, *Science* 278:82-87 (1997), the entirety of which is herein incorporated by reference).

A particularly preferred embodiment of the nucleic acid molecules of the present invention are proteins comprising an amino acid sequence which corresponds to an oxysterol-  
25 protein binding consensus sequence.

In a preferred embodiment of the present invention, the nucleic molecule of the present invention encodes a protein or fragment thereof, where a protein exhibits a BLAST probability



score of greater than 1E-12, preferably a BLAST probability score of between about 1E-30 and about 1E-12, even more preferably a BLAST probability score of greater than 1E-30 with its homologue.

In a preferred embodiment of the present invention, the nucleic molecule of the present invention encodes a protein or fragment thereof where a protein exhibits a BLAST score of greater than 120, preferably a BLAST score of between about 1450 and about 120, even more preferably a BLAST score of greater than 1450 with its homologue.

In another preferred embodiment of the present invention, the nucleic acid molecule encoding a protein or fragment thereof exhibits a % identity with its homologue of between about 25% and about 40%, more preferably of between about 40 and about 70%, even more preferably of between about 70% and about 90% and even more preferably between about 90% and 99%. In another preferred embodiment of the present invention, a protein or fragments thereof exhibits a % identity with its homologue of 100%.

In a preferred embodiment the percent identity calculations are performed using the Megalign program of the LASERGENE bioinformatics computing suite (default parameters, DNASTAR Inc., Madison, Wisconsin).

A protein of the invention can also be a homologue protein. As used herein, a homologue protein molecule or fragment thereof is a counterpart protein molecule or fragment thereof in a second species (*e.g.*, maize HMGC<sub>o</sub>A reductase is a homologue of *Arabidopsis* HMGC<sub>o</sub>A reductase). A homologue can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original (*see*, for example, U.S. Patent 5,811,238, the entirety of which is herein incorporated by reference).

Particularly preferred homologues are selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, maize, peanut, pepper, potato, rice, rye, sorghum, soybean, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce,

lentils, grape, banana, tea, turf grasses, sunflower, soybean, and *Phaseolus*. A particularly preferred group of homologues are those from oil plants such as cotton, canola and sunflower.

In a preferred embodiment, nucleic acid molecules having SEQ ID NO: 1 through SEQ ID NO: 621 or complements and fragments of either can be utilized to obtain such homologues.

5 The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006, the entirety of which is herein incorporated by reference).

Agents of the invention include proteins comprising at least about a 10 amino acid region, more preferably a 20, 30, 40, or 50 amino acid region, of a protein selected from the group  
10 consisting of a fungal, more preferably a yeast HES1, a plant, more preferably a maize, soybean or *Arabidopsis* HES1, a plant, more preferably a rubber or an *Arabidopsis* HMGC<sub>o</sub>A reductase, a plant, more preferably an *Arabidopsis* squalene synthase, a plant, more preferably an *Arabidopsis* cycloartenol synthase, a plant, more preferably an *Arabidopsis* SMTI or SMTII and a fungus, more preferably a yeast UPC2.

### 15 (c) Plant Constructs and Plant Transformants

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of  
20 being inserted into any organism. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the  
25 group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

Genetic material may be transferred into either monocotyledons and dicotyledons including, but not limited to maize, soybean, *Arabidopsis*, phaseolus, peanut, alfalfa, wheat, rice,

oat, sorghum, rye, tritordeum, millet, fescue, perennial ryegrass, sugarcane, cranberry, papaya, banana, muskmelon, apple, cucumber, dendrobium, gladiolus, chrysanthemum, liliacea, cotton, eucalyptus, sunflower, canola, turfgrass, sugarbeet, coffee and dioscorea (Christou, In:

*Particle Bombardment for Genetic Engineering of Plants*, Biotechnology Intelligence Unit,

Academic Press, San Diego, California (1996), the entirety of which is herein incorporated by reference). In a particular preferred embodiment, any seed-bearing plant may be employed as the target plant species for modification in accordance with this invention, including angiosperms, gymnosperms, monocotyledons, and dicotyledons. Plants of special interest are crops harvested for seed oils, including but not limited to rapeseed (high erucic acid rape and canola), maize, soybean, safflower, sunflower, cotton, peanut, flax, oil palm and Cuphea.

Transfer of a nucleic acid that encodes for a protein can result in overexpression of that protein in a transformed cell or transgenic plant. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the invention may be overexpressed in a transformed cell or transformed plant. Such overexpression may be the result of transient or stable transfer of the exogenous genetic material.

In another preferred aspect of the present invention, exogenous genetic material is a nucleic acid molecule that comprises a nucleic acid sequence which encodes a HES1 protein or fragment thereof, more preferably a yeast HES1 protein or fragment thereof, even more preferably a plant HES1 protein or fragment thereof.

In a preferred embodiment, expression or overexpression of a HES1 protein in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of phytosterols.

In a preferred embodiment, expression or overexpression of a HES1 protein in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an altered composition of phytosterols.

In another embodiment, overexpression of a HES1 protein in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of a HES1 protein in a plastid.

In another preferred embodiment, overexpression of the HES1 protein in a transformed plant will result in a plant which as a food or feed constituent exhibits an increased ability to act as a cholesterol lowering agent relative to an untransformed plant with a similar genetic background.

In a preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is selected from the group consisting of a HES1, HMGC<sub>o</sub>A reductase, squalene synthase, cycloartenol synthase, SMTI, SMTII and UPC2. In a more particularly preferred embodiment of the present invention is a protein or fragment thereof, where the protein or fragment thereof is selected from the group consisting of a fungal, more preferably a yeast HES1, a plant, more preferably a maize, soybean or *Arabidopsis* HES1, a plant, more preferably a rubber or an *Arabidopsis* HMGC<sub>o</sub>A reductase, a plant, more preferably an *Arabidopsis* squalene synthase, a plant, more preferably an *Arabidopsis* cycloartenol synthase, a plant, more preferably an *Arabidopsis* SMTI or SMTII and a plant, more preferably a yeast UPC2.

In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is selected from the group consisting a plant HES1, HMGC<sub>o</sub>A reductase, squalene synthase, cycloartenol synthase, SMTI, SMTII and yeast UPC2. In a further even more particularly preferred embodiment of the present invention the protein or fragment thereof is a plant HES1. In an additional even more particularly preferred embodiment of the present invention the protein or fragment thereof is a maize, soybean or *Arabidopsis* HES1.

In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein, preferably a plant HES1 protein comprising an oxysterol-binding protein consensus sequence -- E(K, Q) xSH (H, R) PPx (S, T,

A, C, F)A. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with a conservative amino acid substitution in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with between 2 and 5 conservative amino acid substitutions in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with between 5 and 10 conservative amino acid substitutions in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with more than 10 conservative amino acid substitutions in an amino acid sequence selected from the group consisting of SEQ ID NO: 622, SEQ ID NO: 623, SEQ ID NO: 624 and SEQ ID NO: 625.

In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein that comprises an amino acid sequence SEQ ID NO: 626. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with a conservative amino acid substitution in an amino acid sequence SEQ ID NO: 626. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with between 2 and 5 conservative amino acid substitutions in an amino acid sequence SEQ ID NO: 626. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein

with between 5 and 10 conservative amino acid substitutions in an amino acid sequence SEQ ID NO: 625. In another preferred embodiment of the present invention, the protein or fragment thereof overexpressed in the transgenic plant is a HES1 protein with more than 10 conservative amino acid substitutions in an amino acid sequence SEQ ID NO: 626.

5 Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997), the entirety of which is herein incorporated by reference).

A construct or vector may include a plant promoter to express the protein or protein  
10 fragment of choice. A number of promoters, which are active in plant cells, have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 84:5745-5749 (1987), the entirety of which is herein incorporated by reference), the octopine synthase (OCS) promoter (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al.*, *Plant Mol. Biol.* 9:315-324 (1987), the entirety of which is  
15 herein incorporated by reference) and the CaMV 35S promoter (Odell *et al.*, *Nature* 313:810-812 (1985), the entirety of which is herein incorporated by reference), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*  
20 84:6624-6628 (1987), the entirety of which is herein incorporated by reference), the sucrose synthase promoter (Yang *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:4144-4148 (1990), the entirety of which is herein incorporated by reference), the R gene complex promoter (Chandler *et al.*, *The Plant Cell* 1:1175-1183 (1989), the entirety of which is herein incorporated by reference) and the chlorophyll a/b binding protein gene promoter, etc. These promoters have been used to create  
25 DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913, herein incorporated by reference in its entirety. The CaMV 35S promoters are preferred for use

in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized have relatively high expression in these specific tissues. Tissue-specific expression of a protein of the present invention is a particularly preferred embodiment. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or -enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:3459-3463 (1990), herein incorporated by reference in its entirety), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat (Lloyd *et al.*, *Mol. Gen. Genet.* 225:209-216 (1991), herein incorporated by reference in its entirety), the nuclear photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J.* 8:2445-2451 (1989), herein incorporated by reference in its entirety), the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, *cab6*, from pine (Yamamoto *et al.*, *Plant Cell Physiol.* 35:773-778 (1994), herein incorporated by reference in its entirety), the promoter for the *Cab-1* gene from wheat (Fejes *et al.*, *Plant Mol. Biol.* 15:921-932 (1990), herein incorporated by reference in its entirety), the promoter for the *CAB-1* gene from spinach (Lubberstedt *et al.*, *Plant Physiol.* 104:997-1006 (1994), herein incorporated by reference in its entirety), the promoter for the *cab1R* gene from rice (Luan *et al.*, *Plant Cell.* 4:971-981 (1992), the entirety of which is herein incorporated by reference), the pyruvate, orthophosphate dikinase (PPDK) promoter from maize (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 90: 9586-9590 (1993), herein incorporated by reference in its entirety), the promoter for the tobacco *Lhcb1\*2* gene (Cerdan *et al.*, *Plant Mol. Biol.* 33:245-255 (1997), herein incorporated by reference in its entirety), the *Arabidopsis thaliana* SUC2 sucrose-H<sup>+</sup> symporter promoter (Truernit *et al.*, *Planta.* 196:564-570 (1995), herein incorporated by reference in its

entirety) and the promoter for the thylakoid membrane proteins from spinach (psaD, psaF, psaE, PC, FNR, atpC, atpD, cab, rbcS). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the invention, such as the promoters for LhcB gene and PsbP gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol.* 28:219-229 (1995), the entirety of which is herein incorporated by reference).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of maize, wheat, rice and barley, it is preferred that the promoters utilized in the invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or -enhanced expression are known, including the class I patatin promoter (Bevan *et al.*, *EMBO J.* 8:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol.* 14:995-1006 (1990), both of which are herein incorporated by reference in their entirety), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter (Salanoubat and Belliard, *Gene* 60:47-56 (1987), Salanoubat and Belliard, *Gene* 84:181-185 (1989), both of which are incorporated by reference in their entirety), the promoter for the major tuber proteins including the 22 kd protein complexes and proteinase inhibitors (Hannapel, *Plant Physiol.* 101:703-704 (1993), herein incorporated by reference in its entirety), the promoter for the granule bound starch synthase gene (GBSS) (Visser *et al.*, *Plant Mol. Biol.* 17:691-699 (1991), herein incorporated by reference in its entirety) and other class I and II patatins promoters (Koster-Topfer *et al.*, *Mol Gen Genet.* 219:390-396 (1989); Mignery *et al.*, *Gene.* 62:27-44 (1988), both of which are herein incorporated by reference in their entirety).

Other promoters can also be used to express a protein or fragment thereof in specific tissues, such as seeds or fruits. The promoter for  $\beta$ -conglycinin (Chen *et al.*, *Dev. Genet.* 10: 112-122 (1989), herein incorporated by reference in its entirety) or other seed-specific promoters such as the napin and phaseolin promoters, can be used. The zeins are a group of storage proteins found in maize endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al.*, *Cell* 29:1015-1026 (1982), herein incorporated by reference in its entirety) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and  $\gamma$  genes,



could also be used. Other promoters known to function, for example, in maize include the promoters for the following genes: *waxy*, *Brittle*, *Shrunken 2*, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for maize endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al.*, *Mol. Cell Biol.* 13:5829-5842 (1993), herein incorporated by reference in its entirety). Examples of promoters suitable for expression in wheat include those promoters for the ADPglucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins.

Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al.*, *Plant Mol. Biol.* 25:587-596 (1994), the entirety of which is herein incorporated by reference). Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:7890-7894 (1989), herein incorporated by reference in its entirety). Other root cell specific promoters include those reported by Conkling *et al.* (Conkling *et al.*, *Plant Physiol.* 93:1203-1211 (1990), the entirety of which is herein incorporated by reference).

Additional promoters that may be utilized are described, for example, in U.S. Patent Nos. 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436, all of which are herein incorporated in their entirety. In addition, a

tissue specific enhancer may be used (Fromm *et al.*, *The Plant Cell* 1:977-984 (1989), the entirety of which is herein incorporated by reference).

Constructs or vectors may also include, with the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al.*, *The Plant Cell* 1:671-680 (1989), the entirety of which is herein incorporated by reference; Bevan *et al.*, *Nucleic Acids Res.* 11:369-385 (1983), the entirety of which is herein incorporated by reference).

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop.* 1:1183-1200 (1987), the entirety of which is herein incorporated by reference), the sucrose synthase intron (Vasil *et al.*, *Plant Physiol.* 91:1575-1579 (1989), the entirety of which is herein incorporated by reference) and the TMV omega element (Gallie *et al.*, *The Plant Cell* 1:301-311 (1989), the entirety of which is herein incorporated by reference). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a neo gene (Potrykus *et al.*, *Mol. Gen. Genet.* 199:183-188 (1985), the entirety of which is herein incorporated by reference), which codes for kanamycin resistance and can be selected for using kanamycin, G418, etc.; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology* 6:915-922 (1988), the entirety of which is herein incorporated by reference) which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem.* 263:6310-6314 (1988), the entirety of which is herein incorporated by reference); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985), the entirety of which is herein

incorporated by reference); and a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem.* 263:12500-12508 (1988), the entirety of which is herein incorporated by reference).

A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication Number 0218571, the entirety of which is herein incorporated by reference). Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. For a review of optimizing expression of transgenes, see Koziel *et al.*, *Plant Mol. Biol.* 32:393-405 (1996), the entirety of which is herein incorporated by reference.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include: a  $\beta$ -glucuronidase or uidA gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol. Rep.* 5:387-405 (1987), the entirety of which is herein incorporated by reference; Jefferson *et al.*, *EMBO J.* 6:3901-3907 (1987), the entirety of which is herein incorporated by reference); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, *Stadler Symposium* 11:263-282 (1988), the entirety of which is herein incorporated by reference); a  $\beta$ -lactamase gene (Sutcliffe *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 75:3737-3741 (1978), the entirety of which is herein incorporated by reference), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al.*, *Science* 234:856-859 (1986), the entirety of which is herein incorporated by reference); a xylE gene (Zukowsky *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 80:1101-1105 (1983), the entirety of which is herein incorporated by reference) which encodes a catechol dioxygenase

that can convert chromogenic catechols; an  $\alpha$ -amylase gene (Ikata *et al.*, *Bio/Technol.* 8:241-242 (1990), the entirety of which is herein incorporated by reference); a tyrosinase gene (Katz *et al.*, *J. Gen. Microbiol.* 129:2703-2714 (1983), the entirety of which is herein incorporated by reference) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; an  $\alpha$ -galactosidase, which will turn a chromogenic  $\alpha$ -galactose substrate.

Included within the terms "selectable or screenable marker genes" are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (*e.g.*, by ELISA), small active enzymes which are detectable in extracellular solution (*e.g.*,  $\alpha$ -amylase,  $\beta$ -lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, etc (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:205-225 (1991), the entirety of which is herein incorporated by reference; Vasil, *Plant Mol. Biol.* 25:925-937 (1994), the entirety of which is herein incorporated by reference). For example, electroporation has been used to transform maize protoplasts (Fromm *et al.*, *Nature* 312:791-793 (1986), the entirety of which is herein incorporated by reference).

Other vector systems suitable for introducing transforming DNA into a host plant cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al.*, *Gene* 200:107-116 (1997), the entirety of which is herein incorporated by reference); and transfection with RNA viral vectors (Della-Cioppa *et al.*, *Ann. N.Y. Acad. Sci.* (1996), 792  
5 (Engineering Plants for Commercial Products and Applications), 57-61, the entirety of which is herein incorporated by reference). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al.*, *Molecular Breeding* 4:449-457 (1988), the entirety of which is herein incorporated by reference).

Technology for introduction of DNA into cells is well known to those of skill in the art.  
10 Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973), the entirety of which is herein incorporated by reference); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980), the entirety of which is herein incorporated by reference), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-587 (1982); Fromm *et al.*, *Proc. Natl.*  
15 *Acad. Sci. (U.S.A.)* 82:5824-5828 (1985); U.S. Patent No. 5,384,253, all of which are herein incorporated in their entirety); and the gene gun (Johnston and Tang, *Methods Cell Biol.* 43:353-365 (1994), the entirety of which is herein incorporated by reference); (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques* 6:608-614 (1988), all of which are herein incorporated in their  
20 entirety); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (USA)* 89:6099-6103 (1992), both of which are incorporated by reference in their entirety).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid  
25 molecules to plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press, Oxford, England (1994), the entirety of which is herein incorporated by reference). Non-

biological particles (microprojectiles) that may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts (Cristou *et al.*, *Plant Physiol.* 87:671-674 (1988), the entirety of which is herein incorporated by reference) nor the susceptibility of *Agrobacterium* infection are required. An illustrative embodiment of a method for delivering DNA into maize cells by acceleration is a biolistics  $\alpha$ -particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with maize cells cultured in suspension. Gordon-Kamm *et al.*, describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990), the entirety of which is herein incorporated by reference). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient cells in large aggregates. A particle delivery system suitable for use with the invention is the helium acceleration PDS-1000/He gun is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al.*, *Technique* 3:3-16 (1991), the entirety of which is herein incorporated by reference).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The

number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from one to ten and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include the particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8526-8530 (1990); Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.)* 90:913-917 (1993); Staub and Maliga, *EMBO J.* 12:601-606 (1993); U.S. Patents 5,451,513 and 5,545,818, all of which are herein incorporated by reference in their entirety).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for

optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

*Agrobacterium*-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al.*, *Bio/Technology* 3:629-635 (1985) and Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987), both of which are herein incorporated by reference in their entirety. Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, *Mol. Gen. Genet.* 205:34 (1986), the entirety of which is herein incorporated by reference).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985), the entirety of which is herein incorporated by reference). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is homozygous for the



added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example, Potrykus et al., Mol. Gen. Genet.* 205:193-200 (1986); Lorz *et al., Mol. Gen. Genet.* 199:178 (1985); Fromm *et al., Nature* 319:791 (1986); Uchimiya *et al., Mol. Gen. Genet.* 204:204 (1986); Marcotte *et al., Nature* 335:454-457 (1988), all of which are herein incorporated by reference in their entirety).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue Culture Letters* 2:74 (1985); Toriyama *et al., Theor Appl. Genet.* 205:34 (1986); Yamada *et al., Plant Cell Rep.* 4:85 (1986); Abdullah *et al., Biotechnolog.* 4:1087 (1986), all of which are herein incorporated by reference in their entirety).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, *Biotechnology* 6:397 (1988), the entirety of which is herein incorporated by reference). In addition, "particle

gun" or high-velocity microprojectile technology can be utilized (Vasil *et al.*, *Bio/Technology* 10:667 (1992), the entirety of which is herein incorporated by reference).

Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al.*, *Nature* 328:70 (1987); Klein *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8502-8505 (1988); McCabe *et al.*, *Bio/Technology* 6:923 (1988), all of which are herein incorporated by reference in their entirety). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen (Hess *et al.*, *Intern Rev. Cytol.* 107:367 (1987); Luo *et al.*, *Plant Mol Biol. Reporter* 6:165 (1988), all of which are herein incorporated by reference in their entirety), by direct injection of DNA into reproductive organs of a plant (Pena *et al.*, *Nature* 325:274 (1987), the entirety of which is herein incorporated by reference), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al.*, *Theor. Appl. Genet.* 75:30 (1987), the entirety of which is herein incorporated by reference).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988), the entirety of which is herein incorporated by reference). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the

regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

5           There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

          Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863; U.S. Patent No. 5,159,135; U.S. Patent No. 5,518,908, all of which are herein incorporated by  
10           reference in their entirety); soybean (U.S. Patent No. 5,569,834; U.S. Patent No. 5,416,011; McCabe *et al.*, *Biotechnology* 6:923 (1988); Christou *et al.*, *Plant Physiol.* 87:671-674 (1988); all of which are herein incorporated by reference in their entirety); *Brassica* (U.S. Patent No. 5,463,174, the entirety of which is herein incorporated by reference); peanut (Cheng *et al.*, *Plant Cell Rep.* 15:653-657 (1996), McKently *et al.*, *Plant Cell Rep.* 14:699-703 (1995), all of which  
15           are herein incorporated by reference in their entirety); papaya; and pea (Grant *et al.*, *Plant Cell Rep.* 15:254-258 (1995), the entirety of which is herein incorporated by reference).

          Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been  
20           achieved in asparagus (Bytebier *et al.*, *Proc. Natl. Acad. Sci. (USA)* 84:5354 (1987), the entirety of which is herein incorporated by reference); barley (Wan and Lemaux, *Plant Physiol* 104:37 (1994), the entirety of which is herein incorporated by reference); maize (Rhodes *et al.*, *Science* 240:204 (1988); Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990); Fromm *et al.*, *Bio/Technology* 8:833 (1990); Koziel *et al.*, *Bio/Technology* 11:194 (1993); Armstrong *et al.*,  
25           *Crop Science* 35:550-557 (1995); all of which are herein incorporated by reference in their entirety); oat (Somers *et al.*, *Bio/Technology* 10:1589 (1992), the entirety of which is herein incorporated by reference); orchard grass (Horn *et al.*, *Plant Cell Rep.* 7:469 (1988), the entirety

of which is herein incorporated by reference); rice (Toriyama *et al.*, *Theor Appl. Genet.* 205:34 (1986); Part *et al.*, *Plant Mol. Biol.* 32:1135-1148 (1996); Abedinia *et al.*, *Aust. J. Plant Physiol.* 24:133-141 (1997); Zhang and Wu, *Theor. Appl. Genet.* 76:835 (1988); Zhang *et al.*, *Plant Cell Rep.* 7:379 (1988); Battraw and Hall, *Plant Sci.* 86:191-202 (1992); Christou *et al.*,  
5 *Bio/Technology* 9:957 (1991), all of which are herein incorporated by reference in their entirety);  
rye (De la Pena *et al.*, *Nature* 325:274 (1987), the entirety of which is herein incorporated by  
reference); sugarcane (Bower and Birch, *Plant J.* 2:409 (1992), the entirety of which is herein  
incorporated by reference); tall fescue (Wang *et al.*, *Bio/Technology* 10:691 (1992), the entirety  
of which is herein incorporated by reference) and wheat (Vasil *et al.*, *Bio/Technology* 10:667  
10 (1992), the entirety of which is herein incorporated by reference; U.S. Patent No. 5,631,152, the  
entirety of which is herein incorporated by reference.)

Assays for gene expression based on the transient expression of cloned nucleic acid  
constructs have been developed by introducing the nucleic acid molecules into plant cells by  
polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature*  
15 335:454-457 (1988), the entirety of which is herein incorporated by reference; Marcotte *et al.*,  
*Plant Cell* 1:523-532 (1989), the entirety of which is herein incorporated by reference; McCarty  
*et al.*, *Cell* 66:895-905 (1991), the entirety of which is herein incorporated by reference; Hattori  
*et al.*, *Genes Dev.* 6:609-618 (1992), the entirety of which is herein incorporated by reference;  
Goff *et al.*, *EMBO J.* 9:2517-2522 (1990), the entirety of which is herein incorporated by  
20 reference). Transient expression systems may be used to functionally dissect gene constructs (*see*  
*generally*, Mailga *et al.*, *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995)).

Any of the nucleic acid molecules of the invention may be introduced into a plant cell in a  
permanent or transient manner in combination with other genetic elements such as vectors,  
promoters, enhancers, etc. Further, any of the nucleic acid molecules of the invention may be  
25 introduced into a plant cell in a manner that allows for overexpression of the protein or fragment  
thereof encoded by the nucleic acid molecule.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al.*, *Plant Cell* 2:279-289 (1990), the entirety of which is herein incorporated by reference; van der Krol *et al.*, *Plant Cell* 2:291-299 (1990), the entirety of which is herein incorporated by reference). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Prolls and Meyer, *Plant J.* 2:465-475 (1992), the entirety of which is herein incorporated by reference) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al.*, *Mol. Gen. Genet.* 244:325-330 (1994), the entirety of which is herein incorporated by reference). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, C.R. *Acad. Sci. III* 316:1471-1483 (1993), the entirety of which is herein incorporated by reference).

This technique has, for example, been applied to generate white flowers from red petunia and tomatoes that do not ripen on the vine. Up to 50% of petunia transformants that contained a sense copy of the glucoamylase (CHS) gene produced white flowers or floral sectors; this was as a result of the post-transcriptional loss of mRNA encoding CHS (Flavell, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:3490-3496 (1994), the entirety of which is herein incorporated by reference); van Blokland *et al.*, *Plant J.* 6:861-877 (1994), the entirety of which is herein incorporated by reference). Cosuppression may require the coordinate transcription of the transgene and the endogenous gene and can be reset by a developmental control mechanism (Jorgensen, *Trends Biotechnol.* 8:340-344 (1990), the entirety of which is herein incorporated by reference; Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994), the entirety of which is herein incorporated by reference).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al.*, *FEBS Lett.* 268:427-430 (1990), the entirety of which is herein incorporated by reference). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.*, In: *Genetic Engineering*, Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989), the entirety of which is herein incorporated by reference).

The principle of regulation by antisense RNA is that RNA that is complementary to the target mRNA is introduced into cells, resulting in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al.*, *Annu. Rev. Biochem.* 55:569-597 (1986), the entirety of which is herein incorporated by reference). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol.* 25:155-184 (1990), the entirety of which is herein incorporated by reference). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, etc. The type of transformation and choice of vector will determine

whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule of the present invention whose non-transcribed strand encodes a protein or fragment thereof.

Antibodies have been expressed in plants (Hiatt *et al.*, *Nature* 342:76-78 (1989), the entirety of which is herein incorporated by reference; Conrad and Fielder, *Plant Mol. Biol.* 26:1023-1030 (1994), the entirety of which is herein incorporated by reference). Cytoplasmic expression of a scFv (single-chain Fv antibodies) has been reported to delay infection by artichoke mottled crinkle virus. Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al.*, *EMBO J.* 16:4489-4496 (1997), the entirety of which is herein incorporated by reference; Marion-Poll, *Trends in Plant Science* 2:447-448 (1997), the entirety of which is herein incorporated by reference). For example, expressed anti-abscissic antibodies have been reported to result in a general perturbation of seed development (Philips *et al.*, *EMBO J.* 16: 4489-4496 (1997)).

Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical reaction forward (Persidas, *Nature Biotechnology* 15:1313-1315 (1997), the entirety of which is herein incorporated by reference; Baca *et al.*, *Ann. Rev. Biophys. Biomol. Struct.* 26:461-493 (1997), the entirety of which is herein incorporated by reference). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent No. 5,658,753; U.S. Patent No. 5,632,990; U.S. Patent No. 5,631,137; U.S. Patent 5,602,015; U.S. Patent No. 5,559,538; U.S. Patent No. 5,576,174; U.S. Patent No. 5,500,358; U.S. Patent 5,318,897; U.S. Patent No. 5,298,409; U.S. Patent No. 5,258,289 and U.S. Patent No. 5,194,585, all of which are herein incorporated in their entirety.

It is understood that any of the antibodies of the invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the expressed antibodies may be catalytic.

**(d) Antibodies**

One aspect of the invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the invention and their homologues, fusions or fragments. In a preferred embodiment, an antibody of the present invention binds to an amino acid selected from the group consisting of SEQ ID NO: 622 through 625. Such antibodies may be used to quantitatively or qualitatively detect the protein or peptide molecules of the invention. As used herein, an antibody or peptide is said to “specifically bind” to a protein or peptide molecule of the invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the invention can be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a “fusion” molecule (*i.e.*, a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood that any of the nucleic acid molecules of the invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins fragments (such as (F(ab'), F(ab')<sub>2</sub>), or single-chain immunoglobulins producible, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example,



Harlow and Lane, In: *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988), the entirety of which is herein incorporated by reference).

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 µg of purified protein (or fragment thereof) that has been emulsified in a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)).

Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 µg of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-protein or peptide antibodies. Preferably, a direct binding Enzyme-Linked Immunoassay (ELISA) is employed for this purpose.

More preferably, the mouse having the highest antibody titer is given a third i.v. injection of approximately 25 µg of the same protein or fragment. The splenic leukocytes from this animal may be recovered 3 days later and then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs"), preferably by direct ELISA.

In one embodiment, anti-protein or peptide monoclonal antibodies are isolated using a fusion of a protein or peptide of the invention, or conjugate of a protein or peptide of the invention, as immunogens. Thus, for example, a group of mice can be immunized using a fusion protein emulsified in Freund's complete adjuvant (*e.g.*, approximately 50 µg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If antibody

titers are too low, a fourth booster can be employed. Polysera capable of binding the protein or peptide can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted and immune splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to grow in culture medium supplemented with hypoxanthine, aminopterin and thymidine for approximately 2-3 weeks.

Hybridoma cells that arise from such incubation are preferably screened for their capacity to produce an immunoglobulin that binds to a protein of interest. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example through the use of a chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbor cells. Desirably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred sub-embodiment, a different antigenic form may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one immunogen, but the resulting hybridomas can be screened using a different immunogen. It is understood that any of the protein or peptide molecules of the invention may be used to raise antibodies.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind the protein or peptide molecules of the invention permits the identification of mimetic compounds derived from those molecules. These mimetic compounds may contain a fragment of the protein or peptide or merely a structurally similar region and nonetheless exhibits an ability to specifically bind to antibodies directed  
5 against that compound.

It is understood that any of the agents of the invention can be substantially purified and/or be biologically active and/or recombinant.

#### **(e) Exemplary Uses**

Nucleic acid molecules and fragments thereof of the invention may be employed to obtain  
10 other nucleic acid molecules from the same species (nucleic acid molecules from maize may be utilized to obtain other nucleic acid molecules from maize). Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include  
15 nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the invention may also be employed to obtain nucleic acid homologs. Such homologs include the nucleic acid molecule of other plants  
20 or other organisms (*e.g.*, alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, pea, peanut, pepper, potato, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, oil palm, *Phaseolus*, etc.) including the nucleic acid molecules that encode, in whole or in part, protein homologs of other  
25 plant species or other organisms, sequences of genetic elements, such as promoters and transcriptional regulatory elements. Particularly preferred plants are selected from the group

consisting of maize, canola, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax and sunflower.

Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from such plant species. Methods for forming such libraries are well known in the art. Such homolog molecules may differ in their nucleotide sequences from those found in one or more of SEQ ID NOs: 1-4, 6-29 or complements thereof because complete complementarity is not needed for stable hybridization. The nucleic acid molecules of the invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 83:4143-4146 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5507-5511 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:1028-1032 (1988); Holt *et al.*, *Molec. Cell. Biol.* 8:963-973 (1988); Gerwitz *et al.*, *Science* 242:1303-1306 (1988); Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:3379-3383 (1989); Becker *et al.*, *EMBO J.* 8:3685-3691 (1989)). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki *et al.*, U.S. Patent 4,683,194) to amplify and obtain any desired nucleic acid molecule or fragment.

Promoter sequences and other genetic elements, including but not limited to transcriptional regulatory flanking sequences, associated with one or more of the disclosed nucleic acid sequences can also be obtained using the disclosed nucleic acid sequence provided herein. In one embodiment, such sequences are obtained by incubating nucleic acid molecules of

the present invention with members of genomic libraries and recovering clones that hybridize to such nucleic acid molecules thereof. In a second embodiment, methods of “chromosome walking,” or inverse PCR may be used to obtain such sequences (Frohman *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988); Ohara *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:5673-5677 (1989); Pang *et al.*, *Biotechniques* 22:1046-1048 (1977); Huang *et al.*, *Methods Mol. Biol.* 69:89-96 (1997); Huang *et al.*, *Method Mol. Biol.* 67:287-294 (1997); Benkel *et al.*, *Genet. Anal.* 13:123-127 (1996); Hartl *et al.*, *Methods Mol. Biol.* 58:293-301 (1996)). The term “chromosome walking” means a process of extending a genetic map by successive hybridization steps.

The nucleic acid molecules of the invention may be used to isolate promoters of cell enhanced, cell specific, tissue enhanced, tissue specific, developmentally or environmentally regulated expression profiles. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See, for example, Birren *et al.*, *Genome Analysis: Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Promoters obtained utilizing the nucleic acid molecules of the invention could also be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhancer sequences. Such genetic elements could be used to enhance gene expression of new and existing traits for crop improvement.

Another subset of the nucleic acid molecules of the invention includes nucleic acid molecules that are markers. The markers can be used in a number of conventional ways in the field of molecular genetics. Such markers include nucleic acid molecules SEQ ID NOS: 1-4, 6-29 or complements thereof or fragments of either that can act as markers and other nucleic acid molecules of the present invention that can act as markers.

Genetic markers of the invention include “dominant” or “codominant” markers. “Codominant markers” reveal the presence of two or more alleles (two per diploid individual) at

a locus. “Dominant markers” reveal the presence of only a single allele per locus. The presence of the dominant marker phenotype (e.g., a band of DNA) is an indication that one allele is in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (e.g., absence of a DNA band) is merely evidence that “some other” undefined allele is present. In the case of populations where individuals are predominantly homozygous and loci are predominately dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multi-allelic, codominant markers often become more informative of the genotype than dominant markers. Marker molecules can be, for example, capable of detecting polymorphisms such as single nucleotide polymorphisms (SNPs).

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)). A “polymorphism” is a variation or difference in the sequence of the gene or its flanking regions that arises in some of the members of a species. The variant sequence and the “original” sequence co-exist in the species’ population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be “allelic,” in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e., the original “allele”) whereas other members may have the variant sequence (i.e., the variant “allele”). In the simplest case, only one variant sequence may exist and the polymorphism is thus said to be di-allelic. In other cases, the species’ population may contain multiple alleles and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms

characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity analysis (Weber, U.S. Patent 5,075,217; Armour *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn *et al.*, PCT Patent Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys *et al.*, *Nature* 316:76-79 (1985); Gray *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore *et al.*, *Genomics* 10:654-660 (1991); Jeffreys *et al.*, *Anim. Genet.* 18:1-15 (1987); Hillel *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel *et al.*, *Genet.* 124:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s) and more preferably within 100kb of the polymorphism(s) and most preferably within 10kb of the polymorphism(s) can be employed.

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, organisms that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length

polymorphisms" ("RFLPs") (Glassberg, UK Patent Application 2135774; Skolnick *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer *et al.*, (PCT Application WO90/13668; Uhlen, PCT Application WO90/11369).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996)); Orita *et al.*, *Genomics* 5:874-879 (1989)). A number of protocols have been described for SSCP including, but not limited to, Lee *et al.*, *Anal. Biochem.* 205:289-293 (1992); Suzuki *et al.*, *Anal. Biochem.* 192:82-84 (1991); Lo *et al.*, *Nucleic Acids Research* 20:1005-1009 (1992); Sarkar *et al.*, *Genomics* 13:441-443 (1992). It is understood that one or more of the nucleic acids of the invention, may be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using a DNA fingerprinting technique called amplified fragment length polymorphism (AFLP), which is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA to profile that DNA (Vos *et al.*, *Nucleic Acids Res.* 23:4407-4414 (1995)). This method allows for the specific co-amplification of high numbers of restriction fragments, which can be visualized by PCR without knowledge of the nucleic acid sequence. It is understood that one or more of the nucleic acids of the invention may be utilized as markers or probes to detect polymorphisms by AFLP analysis or for fingerprinting RNA.

Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al.*, *Nucl. Acids Res.* 18:6531-6535 (1990)) and cleaveable amplified polymorphic sequences (CAPS) (Lyamichev *et al.*, *Science* 260:778-783 (1993)). It is understood that one or more of the nucleic acid molecules of the invention, may be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

Single Nucleotide Polymorphisms (SNPs) generally occur at greater frequency than other polymorphic markers and are spaced with a greater uniformity throughout a genome than other reported forms of polymorphism. The greater frequency and uniformity of SNPs means that



there is greater probability that such a polymorphism will be found near or in a genetic locus of interest than would be the case for other polymorphisms. SNPs are located in protein-coding regions and noncoding regions of a genome. Some of these SNPs may result in defective or variant protein expression (e.g., as a result of mutations or defective splicing). Analysis (genotyping) of characterized SNPs can require only a plus/minus assay rather than a lengthy measurement, permitting easier automation.

SNPs can be characterized using any of a variety of methods. Such methods include the direct or indirect sequencing of the site, the use of restriction enzymes (Botstein *et al.*, *Am. J. Hum. Genet.* 32:314-331 (1980), the entirety of which is herein incorporated reference; Konieczny and Ausubel, *Plant J.* 4:403-410 (1993), the entirety of which is herein incorporated by reference), enzymatic and chemical mismatch assays (Myers *et al.*, *Nature* 313:495-498 (1985), the entirety of which is herein incorporated by reference), allele-specific PCR (Newton *et al.*, *Nucl. Acids Res.* 17:2503-2516 (1989), the entirety of which is herein incorporated by reference; Wu *et al.*, *Proc. Natl. Acad. Sci. USA* 86:2757-2760 (1989), the entirety of which is herein incorporated by reference), ligase chain reaction (Barany, *Proc. Natl. Acad. Sci. USA* 88:189-193 (1991), the entirety of which is herein incorporated by reference), single-strand conformation polymorphism analysis (Labrune *et al.*, *Am. J. Hum. Genet.* 48: 1115-1120 (1991), the entirety of which is herein incorporated by reference), single base primer extension (Kuppuswamy *et al.*, *Proc. Natl. Acad. Sci. USA* 88:1143-1147 (1991), Goelet US 6,004,744; Goelet 5,888,819; all of which are herein incorporated by reference in their entirety ), solid-phase ELISA-based oligonucleotide ligation assays (Nikiforov *et al.*, *Nucl. Acids Res.* 22:4167-4175 (1994), dideoxy fingerprinting (Sarkar *et al.*, *Genomics* 13:441-443 (1992), the entirety of which is herein incorporated by reference), oligonucleotide fluorescence-quenching assays (Livak *et al.*, *PCR Methods Appl.* 4:357-362 (1995a), the entirety of which is herein incorporated by reference), 5'-nuclease allele-specific hybridization TaqMan™ assay (Livak *et al.*, *Nature Genet.* 9:341-342 (1995), the entirety of which is herein incorporated by reference), template-directed dye-terminator incorporation (TDI) assay (Chen and Kwok, *Nucl. Acids Res.* 25:347-353 (1997),

the entirety of which is herein incorporated by reference), allele-specific molecular beacon assay (Tyagi *et al.*, *Nature Biotech.* 16: 49-53 (1998), the entirety of which is herein incorporated by reference), PinPoint assay (Haff and Smirnov, *Genome Res.* 7: 378-388 (1997), the entirety of which is herein incorporated by reference), dCAPS analysis (Neff *et al.*, *Plant J.* 14:387-392 (1998), the entirety of which is herein incorporated by reference), pyrosequencing (Ronaghi *et al.*, *Analytical Biochemistry* 267:65-71 (1999); Ronaghi *et al.* PCT application WO 98/13523; Nyren *et al.* PCT application WO 98/28440, all of which are herein incorporated by reference in their entirety; <http://www.pyrosequencing.com>), using mass spectrometry, *e.g.* the Masscode™ system (Howbert *et al.* WO 99/05319; Howber *et al.* WO 97/27331, all of which are herein incorporated by reference in their entirety; <http://www.rapigene.com>; Becker *et al.* PCT application WO 98/26095; Becker *et al.* PCT application; WO 98/12355; Becker *et al.* PCT application WO 97/33000; Monforte *et al.* US 5,965,363, all of which are herein incorporated by reference in their entirety), invasive cleavage of oligonucleotide probes (Lyamichev *et al.* *Nature Biotechnology* 17:292-296, herein incorporated by reference in its entirety; <http://www.twt.com>), and using high density oligonucleotide arrays (Hacia *et al.* *Nature Genetics* 22:164-167; herein incorporated by reference in its entirety; <http://www.affymetrix.com>).

Polymorphisms may also be detected using allele-specific oligonucleotides (ASO), which, can be for example, used in combination with hybridization based technology including southern, northern, and dot blot hybridizations, reverse dot blot hybridizations and hybridizations performed on microarray and related technology.

The stringency of hybridization for polymorphism detection is highly dependent upon a variety of factors, including length of the allele-specific oligonucleotide, sequence composition, degree of complementarity (*i.e.* presence or absence of base mismatches), concentration of salts and other factors such as formamide, and temperature. These factors are important both during the hybridization itself and during subsequent washes performed to remove target polynucleotide that is not specifically hybridized. In practice, the conditions of the final, most stringent wash are most critical. In addition, the amount of target polynucleotide that is able to hybridize to the

allele-specific oligonucleotide is also governed by such factors as the concentration of both the ASO and the target polynucleotide, the presence and concentration of factors that act to “tie up” water molecules, so as to effectively concentrate the reagents (*e.g.*, PEG, dextran, dextran sulfate, *etc.*), whether the nucleic acids are immobilized or in solution, and the duration of hybridization and washing steps.

Hybridizations are preferably performed below the melting temperature ( $T_m$ ) of the ASO. The closer the hybridization and/or washing step is to the  $T_m$ , the higher the stringency.  $T_m$  for an oligonucleotide may be approximated, for example, according to the following formula:  $T_m = 81.5 + 16.6 \times (\log_{10}[\text{Na}^+]) + 0.41 \times (\%G+C) - 675/n$ ; where  $[\text{Na}^+]$  is the molar salt concentration of  $\text{Na}^+$  or any other suitable cation and  $n$  = number of bases in the oligonucleotide. Other formulas for approximating  $T_m$  are available and are known to those of ordinary skill in the art.

Stringency is preferably adjusted so as to allow a given ASO to differentially hybridize to a target polynucleotide of the correct allele and a target polynucleotide of the incorrect allele. Preferably, there will be at least a two-fold differential between the signal produced by the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele (*e.g.*, an ASO specific for a mutant allele cross-hybridizing to a wild-type allele). In more preferred embodiments of the present invention, there is at least a five-fold signal differential. In highly preferred embodiments of the present invention, there is at least an order of magnitude signal differential between the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele.

While certain methods for detecting polymorphisms are described herein, other detection methodologies may be utilized. For example, additional methodologies are known and set forth, in Birren *et al.*, *Genome Analysis*, 4:135-186, *A Laboratory Manual. Mapping Genomes*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999); Maliga *et al.*, *Methods in Plant Molecular Biology. A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold

Spring Harbor, NY (1995); Paterson, *Biotechnology Intelligence Unit: Genome Mapping in Plants*, R.G. Landes Co., Georgetown, TX, and Academic Press, San Diego, CA (1996); *The Maize Handbook*, Freeling and Walbot, eds., Springer-Verlag, New York, NY (1994); *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Elles, ed., Humana Press, Totowa, NJ (1996); Clark, ed., *Plant Molecular Biology: A Laboratory Manual*, Clark, ed., Springer-Verlag, Berlin, Germany (1997), all of which are herein incorporated by reference in their entirety.

Requirements for marker-assisted selection in a plant breeding program are: (1) the marker(s) should co-segregate or be closely linked with the desired trait; (2) an efficient means of screening large populations for the molecular marker(s) should be available; and (3) the screening technique should have high reproducibility across laboratories and preferably be economical to use and be user-friendly.

The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics* 121:185-199 (1989) and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics* 121:185-199 (1989) and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL*, Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A  $\log_{10}$  of an odds ratio (LOD) is then calculated as:  $\text{LOD} = \log_{10}(\text{MLE for the presence of a QTL} / \text{MLE given no linked QTL})$ .

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a

false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein, *Genetics* 121:185-199 (1989) and further described by Arús and Moreno-González, *Plant Breeding*, Hayward *et al.*, (eds.) Chapman & Hall, London, pp. 314-331 (1993).

5 In a preferred embodiment of the present invention the nucleic acid marker exhibits a LOD score of greater than 2.0, more preferably 2.5, even more preferably greater than 3.0 or 4.0 with the trait or phenotype of interest. In a preferred embodiment, the trait of interest is altered, preferably increased phytosterol levels or compositions.

Additional models can be used. Many modifications and alternative approaches to  
10 interval mapping have been reported, including the use non-parametric methods (Kruglyak and Lander, *Genetics* 139:1421-1428 (1995)). Multiple regression methods or models can be also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.), Proceedings of the Ninth Meeting of the Eucarpia Section  
15 Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics* 136:1447-1455 (1994), and Zeng, *Genetics* 136:1457-1468 (1994) . Generally, the use of cofactors reduces the bias and sampling error of  
20 the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics* 136:1457-1468 (1994), herein incorporated by reference in its entirety). These models can be extended to multi-environment experiments to analyze genotype-  
25 environment interactions (Jansen *et al.*, *Theo. Appl. Genet.* 91:33-37 (1995), herein incorporated by reference in its entirety).

It is understood that one or more of the nucleic acid molecules of the invention may be used as molecular markers. It is also understood that one or more of the protein molecules of the invention may be used as molecular markers.

In a preferred embodiment, the polymorphism is present and screened for in a mapping population, *e.g.* a collection of plants capable of being used with markers such as polymorphic markers to map genetic position of traits. The choice of appropriate mapping population often depends on the type of marker systems employed (Tanksley *et al.*, *J.P. Gustafson and R. Appels* (eds.). Plenum Press, New York, pp. 157-173 (1988), the entirety of which is herein incorporated by reference). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large number of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An  $F_2$  population is the first generation of selfing (self-pollinating) after the hybrid seed is produced. Usually a single  $F_1$  plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) pattern. Maximum genetic information is obtained from a completely classified  $F_2$  population using a codominant marker system (Mather, *Measurement of Linkage in Heredity*: Methuen and Co., (1938), the entirety of which is herein incorporated by reference). In the case of dominant markers, progeny tests (*e.g.*,  $F_3$ ,  $BCF_2$ ) are required to identify the heterozygotes, in order to classify the population. However, this procedure is often prohibitive because of the cost and time involved in progeny testing. Progeny testing of  $F_2$  individuals is often used in map construction where phenotypes do not consistently reflect genotype (*e.g.* disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations *e.g.*  $F_3$  or  $BCF_2$ ) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations ( $F_2$ ,  $F_3$ ), where linkage groups have not been completely disassociated by recombination events (*i.e.*, maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually  $>F_5$ , developed from continuously selfing  $F_2$  lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about  $<10\%$  recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter. *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992), the entirety of which is herein incorporated by reference). However, as the distance between markers becomes larger (*i.e.*, loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992), the entirety of which is herein incorporated by reference). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from  $F_2$  populations because one, rather than two, recombinant gamete is sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (*i.e.* about  $.15\%$  recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) (created by many backcrosses to produce a collection of individuals that is nearly identical in genetic composition except for the trait or genomic region

under interrogation) can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci is expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88:9828-9832 (1991), the entirety of which is herein incorporated by reference). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular disease) or genomic region but arbitrary at unlinked regions (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

In an aspect of the present invention, one or more of the nucleic molecules of the present invention are used to determine the level (*i.e.*, the concentration of mRNA in a sample, *etc.*) in a plant (preferably maize, canola, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax or sunflower) or pattern (*i.e.*, the kinetics of expression, rate of decomposition, stability profile, *etc.*) of the expression of a protein encoded in part or whole by one or more of the nucleic acid molecule of the present invention (collectively, the "Expression Response" of a cell or tissue).

As used herein, the Expression Response manifested by a cell or tissue is said to be "altered" if it differs from the Expression Response of cells or tissues of plants not exhibiting the phenotype. To determine whether a Expression Response is altered, the Expression Response manifested by the cell or tissue of the plant exhibiting the phenotype is compared with that of a similar cell or tissue sample of a plant not exhibiting the phenotype. As will be appreciated, it is not necessary to re-determine the Expression Response of the cell or tissue sample of plants not exhibiting the phenotype each time such a comparison is made; rather, the Expression Response of a particular plant may be compared with previously obtained values of normal plants. As used herein, the phenotype of the organism is any of one or more characteristics of an organism (*e.g.* disease resistance, pest tolerance, environmental tolerance such as tolerance to abiotic stress,



male sterility, quality improvement or yield *etc.*). A change in genotype or phenotype may be transient or permanent. Also as used herein, a tissue sample is any sample that comprises more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g. derived from root, seed, flower, leaf, stem or pollen *etc.*).

5 In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid molecules of the present invention are utilized to detect the presence or quantity of the mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid  
10 molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative assay that  
15 indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

A number of methods can be used to compare the expression response between two or more samples of cells or tissue. These methods include hybridization assays, such as northern, RNase protection assays, and *in situ* hybridization. Alternatively, the methods include PCR-type assays. In a preferred method, the expression response is compared by hybridizing nucleic acids  
20 from the two or more samples to an array of nucleic acids. The array contains a plurality of suspected sequences known or suspected of being present in the cells or tissue of the samples.

An advantage of *in situ* hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer *et al.*, *Dev. Biol.* 101:477-484 (1984); Angerer *et al.*, *Dev. Biol.* 112:157-166 (1985);  
25 Dixon *et al.*, *EMBO J.* 10:1317-1324 (1991)). *In situ* hybridization may be used to measure the steady-state level of RNA accumulation (Hardin *et al.*, *J. Mol. Biol.* 202:417-431 (1989)). A number of protocols have been devised for *in situ* hybridization, each with tissue preparation,

hybridization and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep.* 5:242-250 (1987); Cox and Goldberg, In: *Plant Molecular Biology: A Practical Approach*, Shaw (ed.), pp. 1-35, IRL Press, Oxford (1988); Raikhel *et al.*, *In situ RNA hybridization in plant tissues*, In: *Plant Molecular Biology Manual*, vol. B9:1-32, Kluwer Academic Publisher, Dordrecht, Belgium (1989)).

*In situ* hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization*, Oxford University Press, Oxford (1992); Langdale, *In Situ Hybridization* In: *The Maize Handbook*, Freeling and Walbot (eds.), pp. 165-179, Springer-Verlag, New York (1994)). It is understood that one or more of the molecules of the invention, preferably one or more of the nucleic acid molecules or fragments thereof of the invention or one or more of the antibodies of the invention may be utilized to detect the level or pattern of a protein or mRNA thereof by *in situ* hybridization.

Fluorescent *in situ* hybridization allows the localization of a particular DNA sequence along a chromosome, which is useful, among other uses, for gene mapping, following chromosomes in hybrid lines, or detecting chromosomes with translocations, transversions or deletions. *In situ* hybridization has been used to identify chromosomes in several plant species (Griffor *et al.*, *Plant Mol. Biol.* 17:101-109 (1991); Gustafson *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:1899-1902 (1990); Mukai and Gill, *Genome* 34:448-452 (1991); Schwarzacher and Heslop-Harrison, *Genome* 34:317-323 (1991); Wang *et al.*, *Jpn. J. Genet.* 66:313-316 (1991); Parra and Windle, *Nature Genetics* 5:17-21 (1993)). It is understood that the nucleic acid molecules of the invention may be used as probes or markers to localize sequences along a chromosome.

Another method to localize the expression of a molecule is tissue printing. Tissue printing provides a way to screen, at the same time on the same membrane many tissue sections from different plants or different developmental stages (Yomo and Taylor, *Planta* 112:35-43 (1973); Harris and Chrispeels, *Plant Physiol.* 56:292-299 (1975); Cassab and Varner, *J. Cell. Biol.* 105:2581-2588 (1987); Spruce *et al.*, *Phytochemistry* 26:2901-2903 (1987); Barres *et al.*,

*Neuron* 5:527-544 (1990); Reid and Pont-Lezica, *Tissue Printing: Tools for the Study of Anatomy, Histochemistry and Gene Expression*, Academic Press, New York, New York (1992); Reid *et al.*, *Plant Physiol.* 93:160-165 (1990); Ye *et al.*, *Plant J.* 1:175-183 (1991)).

A microarray-based method for high-throughput monitoring of gene expression may be  
5 utilized to measure expression response. This 'chip'-based approach involves microarrays of  
nucleic acid molecules as gene-specific hybridization targets to quantitatively measure  
expression of the corresponding mRNA (Schena *et al.*, *Science* 270:467-470 (1995), the entirety  
of which is herein incorporated by reference; <http://cmgm.stanford.edu/pbrown/array.html>;  
Shalon, Ph.D. Thesis, Stanford University (1996), the entirety of which is herein incorporated by  
10 reference). Hybridization to a microarray can be used to efficiently analyze the presence and/or  
amount of a number of nucleotide sequences simultaneously.

Several microarray methods have been described. One method compares the sequences  
to be analyzed by hybridization to a set of oligonucleotides representing all possible  
subsequences (Bains and Smith, *J. Theor. Biol.* 135:303-307 (1989), the entirety of which is  
15 herein incorporated by reference). A second method hybridizes the sample to an array of  
oligonucleotide or cDNA molecules. An array consisting of oligonucleotides complementary to  
subsequences of a target sequence can be used to determine the identity of a target sequence,  
measure its amount, and detect single nucleotide differences between the target and a reference  
sequence. Nucleic acid molecule microarrays may also be screened with protein molecules or  
20 fragments thereof to determine nucleic acid molecules that specifically bind protein molecules or  
fragments thereof.

The microarray approach may be used with polypeptide targets (U.S. Patent No.  
5,445,934; U.S. Patent No: 5,143,854; U.S. Patent No. 5,079,600; U.S. Patent No. 4,923,901, all  
of which are herein incorporated by reference in their entirety). Essentially, polypeptides are  
25 synthesized on a substrate (microarray) and these polypeptides can be screened with either  
protein molecules or fragments thereof or nucleic acid molecules in order to screen for either  
protein molecules or fragments thereof or nucleic acid molecules that specifically bind the target

polypeptides. (Fodor *et al.*, *Science* 251:767-773 (1991), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acid molecules or protein or fragments thereof of the invention may be utilized in a microarray-based method.

In a preferred embodiment of the present invention microarrays may be prepared that  
5 comprise nucleic acid molecules where preferably at least 10%, preferably at least 25%, more preferably at least 50% and even more preferably at least 75%, 80%, 85%, 90% or 95% of the nucleic acid molecules located on that array are selected from the group of nucleic acid molecules that specifically hybridize to one or more nucleic acid molecule having a nucleic acid sequence selected from the group of SEQ ID NO: 1 through SEQ ID NO: 621 or complements  
10 thereof or fragments of either.

In another preferred embodiment of the present invention microarrays may be prepared that comprise nucleic acid molecules where preferably at least 10%, preferably at least 25%, more preferably at least 50% and even more preferably at least 75%, 80%, 85%, 90% or 95% of the nucleic acid molecules located on that array are selected from the group of nucleic acid  
15 molecules having a nucleic acid sequence selected from the group of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either.

In a preferred embodiment of the present invention microarrays may be prepared that comprise nucleic acid molecules where such nucleic acid molecules encode at least one, preferably at least two, more preferably at least three, even more preferably at least four, five or  
20 six proteins or fragments thereof selected from the group consisting of HES1, HMGC<sub>o</sub>A reductase, squalene synthase, cycloartenol synthase, SMTII and UPC2. In even more preferred embodiment of the present invention microarrays may be prepared that comprise nucleic acid molecules where such nucleic acid molecules encode at least one, preferably at least two, more preferably at least three, even more preferably at least four, five or six proteins or fragments  
25 thereof selected from the group consisting of a fungal, more preferably a yeast HES1, a plant, more preferably a maize, soybean or *Arabidopsis* HES1, a plant, more preferably a rubber or an *Arabidopsis* HMGC<sub>o</sub>A reductase, a plant, more preferably an *Arabidopsis* squalene synthase, a

plant, more preferably an *Arabidopsis* cycloartenol synthase, a plant, more preferably an *Arabidopsis* SMTII and a fungus, more preferably a yeast UPC2.

Site directed mutagenesis may be utilized to modify nucleic acid sequences, particularly as it is a technique that allows one or more of the amino acids encoded by a nucleic acid molecule to be altered (*e.g.*, a threonine to be replaced by a methionine). At least three basic methods for site directed mutagenesis can be employed. These are cassette mutagenesis (Wells *et al.*, *Gene* 34:315-323 (1985), the entirety of which is herein incorporated by reference), primer extension (Gilliam *et al.*, *Gene* 12:129-137 (1980), the entirety of which is herein incorporated by reference; Zoller and Smith, *Methods Enzymol.* 100:468-500 (1983), the entirety of which is herein incorporated by reference; Dalbadie-McFarland *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 79:6409-6413 (1982), the entirety of which is herein incorporated by reference) and methods based upon PCR (Scharf *et al.*, *Science* 233:1076-1078 (1986), the entirety of which is herein incorporated by reference; Higuchi *et al.*, *Nucleic Acids Res.* 16:7351-7367 (1988), the entirety of which is herein incorporated by reference). Site directed mutagenesis approaches are also described in U.S. Patent 5,811,238, European Patent 0 385 962, the entirety of which is herein incorporated by reference; European Patent 0 359 472, the entirety of which is herein incorporated by reference; and PCT Patent Application WO 93/07278, the entirety of which is herein incorporated by reference.

Site directed mutagenesis strategies have been applied to plants for both *in vitro* as well as *in vivo* site directed mutagenesis (Lanz *et al.*, *J. Biol. Chem.* 266:9971-9976 (1991), the entirety of which is herein incorporated by reference; Kovgan and Zhdanov, *Biotekhnologiya* 5:148-154, No. 207160n, Chemical Abstracts 110:225 (1989), the entirety of which is herein incorporated by reference; Ge *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:4037-4041 (1989), the entirety of which is herein incorporated by reference; Zhu *et al.*, *J. Biol. Chem.* 271:18494-18498 (1996), the entirety of which is herein incorporated by reference; Chu *et al.*, *Biochemistry* 33:6150-6157 (1994), the entirety of which is herein incorporated by reference; Small *et al.*, *EMBO J.* 11:1291-1296 (1992), the entirety of which is herein incorporated by reference; Cho *et*

al., *Mol. Biotechnol.* 8:13-16 (1997), the entirety of which is herein incorporated by reference; Kita *et al.*, *J. Biol. Chem.* 271:26529-26535 (1996), the entirety of which is herein incorporated by reference; Jin *et al.*, *Mol. Microbiol.* 7:555-562 (1993), the entirety of which is herein incorporated by reference; Hatfield and Vierstra, *J. Biol. Chem.* 267:14799-14803 (1992), the entirety of which is herein incorporated by reference; Zhao *et al.*, *Biochemistry* 31:5093-5099 (1992), the entirety of which is herein incorporated by reference).

Any of the nucleic acid molecules of the invention may either be modified by site directed mutagenesis or used as, for example, nucleic acid molecules that are used to target other nucleic acid molecules for modification. It is understood that mutants with more than one altered nucleotide can be constructed using techniques that practitioners are familiar with, such as isolating restriction fragments and ligating such fragments into an expression vector (*see*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989)).

Sequence-specific DNA-binding proteins play a role in the regulation of transcription. The isolation of recombinant cDNAs encoding these proteins facilitates the biochemical analysis of their structural and functional properties. Genes encoding such DNA-binding proteins have been isolated using classical genetics (Vollbrecht *et al.*, *Nature* 350: 241-243 (1991), the entirety of which is herein incorporated by reference) and molecular biochemical approaches, including the screening of recombinant cDNA libraries with antibodies (Landschulz *et al.*, *Genes Dev.* 2:786-800 (1988), the entirety of which is herein incorporated by reference) or DNA probes (Bodner *et al.*, *Cell* 55:505-518 (1988), the entirety of which is herein incorporated by reference). In addition, an *in situ* screening procedure has been used and has facilitated the isolation of sequence-specific DNA-binding proteins from various plant species (Gilmartin *et al.*, *Plant Cell* 4:839-849 (1992), the entirety of which is herein incorporated by reference; Schindler *et al.*, *EMBO J.* 11:1261-1273 (1992), the entirety of which is herein incorporated by reference). An *in situ* screening protocol does not require the purification of the protein of interest (Vinson *et al.*,

*Genes Dev.* 2:801-806 (1988), the entirety of which is herein incorporated by reference; Singh *et al.*, *Cell* 52:415-423 (1988), the entirety of which is herein incorporated by reference).

Two steps may be employed to characterize DNA-protein interactions. The first is to identify sequence fragments that interact with DNA-binding proteins, to titrate binding activity, to determine the specificity of binding and to determine whether a given DNA-binding activity can interact with related DNA sequences (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)). Electrophoretic mobility-shift assay is a widely used assay. The assay provides a rapid and sensitive method for detecting DNA-binding proteins based on the observation that the mobility of a DNA fragment through a nondenaturing, low-ionic strength polyacrylamide gel is retarded upon association with a DNA-binding protein (Fried and Crother, *Nucleic Acids Res.* 9:6505-6525 (1981), the entirety of which is herein incorporated by reference). When one or more specific binding activities have been identified, the exact sequence of the DNA bound by the protein may be determined.

Several procedures for characterizing protein/DNA-binding sites are used, including methylation and ethylation interference assays (Maxam and Gilbert, *Methods Enzymol.* 65:499-560 (1980), the entirety of which is herein incorporated by reference; Wissman and Hillen, *Methods Enzymol.* 208:365-379 (1991), the entirety of which is herein incorporated by reference), footprinting techniques employing DNase I (Galas and Schmitz, *Nucleic Acids Res.* 5:3157-3170 (1978), the entirety of which is herein incorporated by reference), 1,10-phenanthroline-copper ion methods (Sigman *et al.*, *Methods Enzymol.* 208:414-433 (1991), the entirety of which is herein incorporated by reference) and hydroxyl radicals methods (Dixon *et al.*, *Methods Enzymol.* 208:414-433 (1991), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acid molecules of the invention may be utilized to identify a protein or fragment thereof that specifically binds to a nucleic acid molecule of the invention. It is also understood that one or more of the protein molecules or

fragments thereof of the invention may be utilized to identify a nucleic acid molecule that specifically binds to it.

A two-hybrid system is based on the fact that proteins, such as transcription factors that interact (physically) with one another carry out many cellular functions. Two-hybrid systems have been used to probe the function of new proteins (Chien *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9578-9582 (1991) the entirety of which is herein incorporated by reference; Durfee *et al.*, *Genes Dev.* 7:555-569 (1993) the entirety of which is herein incorporated by reference; Choi *et al.*, *Cell* 78:499-512 (1994), the entirety of which is herein incorporated by reference; Kranz *et al.*, *Genes Dev.* 8:313-327 (1994), the entirety of which is herein incorporated by reference).

Interaction mating techniques have facilitated a number of two-hybrid studies of protein-protein interaction. Interaction mating has been used to examine interactions between small sets of tens of proteins (Finley and Brent, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:12098-12984 (1994), the entirety of which is herein incorporated by reference), larger sets of hundreds of proteins (Bendixen *et al.*, *Nucl. Acids Res.* 22:1778-1779 (1994), the entirety of which is herein incorporated by reference) and to comprehensively map proteins encoded by a small genome (Bartel *et al.*, *Nature Genetics* 12:72-77 (1996), the entirety of which is herein incorporated by reference). This technique utilizes proteins fused to the DNA-binding domain and proteins fused to the activation domain. They are expressed in two different haploid yeast strains of opposite mating type and the strains are mated to determine if the two proteins interact. Mating occurs when haploid yeast strains come into contact and result in the fusion of the two haploids into a diploid yeast strain. An interaction can be determined by the activation of a two-hybrid reporter gene in the diploid strain.

The CLONTECH laboratories, Inc. provides the MATCHMAKER two-hybrid System kit (Cat. No. K1605-1) in which the sequences encoding the two functional domains of the GAL4 transcriptional activator, DNA binding domain and activation domain, are cloned into two different shuttle/expression vectors (pGBT9 and pGAD424) ( Bartel *et al. In Cellular Interactions in Development: A Practical Approach*, D.A. Hartley, ed., Oxford University Press,



Oxford 153-179 (1993), the entirety of which is herein incorporated by reference). The gene code for the target protein is cloned into the pGBT9 to generate a hybrid of GAL4-DNA binding domain with a target protein and the gene(s) encode for potentially interacting protein(s) are cloned into the pGAD424 to create hybrid protein(s) of GAL4-activation domain with potentially interacting protein or with a collection of random proteins in a fusion library. The both plasmids carrying hybrid proteins are cotransformed into one yeast strain. Both hybrid proteins are targeted to the yeast nucleus by nuclear localization signal. If the target protein and the potentially interacting protein interact with each other, the GAL4 DNA binding domain and the GAL4 activation domain are brought to proximity and proper function of the transcriptional activator unit will be reconstituted resulting in transcription of reporter gene (lacZ or HIS3). An advantage of this technique is that it reduces the number of yeast transformations needed to test individual interactions. It is understood that the protein-protein interactions of protein or fragments thereof of the invention may be investigated using the two-hybrid system and that any of the nucleic acid molecules of the invention that encode such proteins or fragments thereof may be used to transform yeast in the two-hybrid system.

**(f) Fungal Constructs and Fungal Transformants**

The invention also relates to a fungal recombinant vector comprising exogenous genetic material. The invention also relates to a fungal cell comprising a fungal recombinant vector. The invention also relates to methods for obtaining a recombinant fungal host cell comprising introducing into a fungal host cell exogenous genetic material.

Exogenous genetic material may be transferred into a fungal cell. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

The fungal recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures. The choice of a vector will typically depend on the compatibility of the vector with the fungal host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the fungal host.

The fungal vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the fungal cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. This integration may be the result of homologous or non-homologous recombination.

Integration of a vector or nucleic acid into the genome by homologous recombination, regardless of the host being considered, relies on the nucleic acid sequence of the vector. Typically, the vector contains nucleic acid sequences for directing integration by homologous recombination into the genome of the host. These nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location or locations in one or more chromosomes. To increase the likelihood of integration at a precise location, there should be preferably two nucleic acid sequences that individually contain a sufficient number of nucleic acids, preferably 400bp to 1500bp, more preferably 800bp to 1000bp, which are highly homologous with the corresponding host cell target sequence. This enhances the probability of homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a host cell target sequence and, furthermore, may or may not encode proteins.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication and the combination

of CEN3 and ARS 1. Any origin of replication may be used which is compatible with the fungal host cell of choice.

The fungal vectors of the invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides, for example biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs and the like. The selectable marker may be selected from the group including, but not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase) and *sC* (sulfate adenylyltransferase) and *trpC* (anthranilate synthase). Preferred for use in an *Aspergillus* cell are the *amdS* and *pyrG* markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* marker of *Streptomyces hygrosopicus*. Furthermore, selection may be accomplished by co-transformation, *e.g.*, as described in WO 91/17243, the entirety of which is herein incorporated by reference. A nucleic acid sequence of the invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleic acid sequence which is recognized by the fungal host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the protein or fragment thereof.

A promoter may be any nucleic acid sequence which shows transcriptional activity in the fungal host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of a nucleic acid construct of the invention in a filamentous fungal host are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase and hybrids thereof. In a yeast host, a useful promoter is the *Saccharomyces cerevisiae* enolase (*eno-1*) promoter. Particularly preferred

promoters are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the genes encoding *Aspergillus niger* neutral alpha -amylase and *Aspergillus oryzae* triose phosphate isomerase), glaA, *Saccharomyces cerevisiae* GAL1 (galactokinase) and *Saccharomyces cerevisiae* GPD (glyceraldehyde-3-phosphate dehydrogenase) promoters.

5 A protein or fragment thereof encoding nucleic acid molecule of the invention may also be operably linked to a terminator sequence at its 3' terminus. The terminator sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any terminator which is functional in the fungal host cell of choice may be used in the invention, but particularly preferred terminators are obtained from the genes  
10 encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase, *Saccharomyces cerevisiae* cytochrome-c oxidase (CYC1) and *Saccharomyces cerevisiae* enolase.

A protein or fragment thereof encoding nucleic acid molecule of the invention may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated region of a  
15 mRNA which is important for translation by the fungal host. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the protein or fragment thereof. The leader sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence which is functional in the fungal host cell of choice may be used in the invention, but particularly preferred leaders are  
20 obtained from the genes encoding *Aspergillus oryzae* TAKA amylase and *Aspergillus oryzae* triose phosphate isomerase.

A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleic acid sequence of the invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the fungal host to add polyadenosine residues to transcribed mRNA.  
25 The polyadenylation sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence which is functional in the fungal host of choice may be used in the invention, but particularly preferred

polyadenylation sequences are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase and *Saccharomyces cerevisiae* cytochrome-c oxidase (CYC1).

To avoid the necessity of disrupting the cell to obtain the protein or fragment thereof and to minimize the amount of possible degradation of the expressed protein or fragment thereof within the cell, it is preferred that expression of the protein or fragment thereof gives rise to a product secreted outside the cell. To this end, a protein or fragment thereof of the invention may be linked to a signal peptide linked to the amino terminus of the protein or fragment thereof. A signal peptide is an amino acid sequence which permits the secretion of the protein or fragment thereof from the fungal host into the culture medium. The signal peptide may be native to the protein or fragment thereof of the invention or may be obtained from foreign sources. The 5' end of the coding sequence of the nucleic acid sequence of the invention may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted protein or fragment thereof. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to that portion of the coding sequence which encodes the secreted protein or fragment thereof. The foreign signal peptide may be required where the coding sequence does not normally contain a signal peptide coding region. Alternatively, the foreign signal peptide may simply replace the natural signal peptide to obtain enhanced secretion of the desired protein or fragment thereof. The foreign signal peptide coding region may be obtained from a glucoamylase or an amylase gene from an *Aspergillus* species, a lipase or proteinase gene from *Rhizomucor miehei*, the gene for the alpha-factor from *Saccharomyces cerevisiae*, or the calf preprochymosin gene. An effective signal peptide for fungal host cells is the *Aspergillus oryzae* TAKA amylase signal, *Aspergillus niger* neutral amylase signal, the *Rhizomucor miehei* aspartic proteinase signal, the *Humicola lanuginosus* cellulase signal, or the *Rhizomucor miehei* lipase signal. However, any signal peptide capable of permitting secretion of the protein or fragment thereof in a fungal host of choice may be used in the invention.

A protein or fragment thereof encoding nucleic acid molecule of the invention may also be linked to a propeptide coding region. A propeptide is an amino acid sequence found at the amino terminus of a proprotein or proenzyme. Cleavage of the propeptide from the proprotein yields a mature biochemically active protein. The resulting polypeptide is known as a propolypeptide or proenzyme (or a zymogen in some cases). Propolypeptides are generally inactive and can be converted to mature active polypeptides by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide or proenzyme. The propeptide coding region may be native to the protein or fragment thereof or may be obtained from foreign sources. The foreign propeptide coding region may be obtained from the *Saccharomyces cerevisiae* alpha-factor gene or *Myceliophthora thermophila* laccase gene (WO 95/33836, the entirety of which is herein incorporated by reference).

The procedures used to ligate the elements described above to construct the recombinant expression vector of the invention are well known to one skilled in the art (see, for example, Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor, N.Y., (1989)).

The invention also relates to recombinant fungal host cells produced by the methods of the invention which are advantageously used with the recombinant vector of the invention. The cell is preferably transformed with a vector comprising a nucleic acid sequence of the invention followed by integration of the vector into the host chromosome. The choice of fungal host cells will to a large extent depend upon the gene encoding the protein or fragment thereof and its source. The fungal host cell may, for example, be a yeast cell or a filamentous fungal cell.

"Yeast" as used herein includes *Ascosporogenous* yeast (*Endomycetales*), *Basidiosporogenous* yeast and yeast belonging to the *Fungi Imperfecti* (*Blastomycetes*). The *Ascosporogenous* yeasts are divided into the families *Spermophthoraceae* and *Saccharomycetaceae*. The latter is comprised of four subfamilies, *Schizosaccharomycoideae* (for example, genus *Schizosaccharomyces*), *Nadsonioideae*, *Lipomycoideae* and *Saccharomycoideae* (for example, genera *Pichia*, *Kluyveromyces* and *Saccharomyces*). The *Basidiosporogenous*

yeasts include the genera *Leucosporidium*, *Rhodospodium*, *Sporidiobolus*, *Filobasidium* and *Filobasidiella*. Yeast belonging to the *Fungi Imperfecti* are divided into two families, *Sporobolomycetaceae* (for example, genera *Sorobolomyces* and *Bullera*) and *Cryptococcaceae* (for example, genus *Candida*). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner *et al.*, *Soc. App. Bacteriol. Symposium Series* No. 9, (1980), the entirety of which is herein incorporated by reference). The biology of yeast and manipulation of yeast genetics are well known in the art (*see*, for example, *Biochemistry and Genetics of Yeast*, Bacil *et al.* (ed.), 2nd edition, 1987; *The Yeasts*, Rose and Harrison (eds.), 2nd ed., (1987); and *The Molecular Biology of the Yeast Saccharomyces*, Strathern *et al.* (eds.), (1981), all of which are herein incorporated by reference in their entirety).

"Fungi" as used herein includes the phyla *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Zygomycota* (as defined by Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8<sup>th</sup> edition, 1995, CAB International, University Press, Cambridge, UK; the entirety of which is herein incorporated by reference) as well as the *Oomycota* (as cited in Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8<sup>th</sup> edition, 1995, CAB International, University Press, Cambridge, UK) and all mitosporic fungi (Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8<sup>th</sup> edition, 1995, CAB International, University Press, Cambridge, UK). Representative groups of *Ascomycota* include, for example, *Neurospora*, *Eupenicillium* (= *Penicillium*), *Emericella* (= *Aspergillus*), *Eurotium* (= *Aspergillus*) and the true yeasts listed above. Examples of *Basidiomycota* include mushrooms, rusts and smuts. Representative groups of *Chytridiomycota* include, for example, *Allomyces*, *Blastocladiella*, *Coelomomyces* and aquatic fungi. Representative groups of *Oomycota* include, for example, *Saprolegniomycetous* aquatic fungi (water molds) such as *Achlya*. Examples of mitosporic fungi include *Aspergillus*, *Penicillium*, *Candida* and *Alternaria*. Representative groups of *Zygomycota* include, for example, *Rhizopus* and *Mucor*.

"Filamentous fungi" include all filamentous forms of the subdivision *Eumycota* and *Oomycota* (as defined by Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8<sup>th</sup> edition, 1995, CAB International, University Press, Cambridge, UK). The filamentous fungi are characterized by a vegetative mycelium composed of chitin, cellulose, glucan, chitosan, mannan and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

In one embodiment, the fungal host cell is a yeast cell. In a preferred embodiment, the yeast host cell is a cell of the species of *Candida*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Pichia* and *Yarrowia*. In a preferred embodiment, the yeast host cell is a *Saccharomyces cerevisiae* cell, a *Saccharomyces carlsbergensis*, *Saccharomyces diastaticus* cell, a *Saccharomyces douglasii* cell, a *Saccharomyces kluyveri* cell, a *Saccharomyces norbensis* cell, or a *Saccharomyces oviformis* cell. In another preferred embodiment, the yeast host cell is a *Kluyveromyces lactis* cell. In another preferred embodiment, the yeast host cell is a *Yarrowia lipolytica* cell.

In another embodiment, the fungal host cell is a filamentous fungal cell. In a preferred embodiment, the filamentous fungal host cell is a cell of the species of, but not limited to, *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Myceliophthora*, *Mucor*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium* and *Trichoderma*.

The recombinant fungal host cells of the invention may further comprise one or more sequences which encode one or more factors that are advantageous in the expression of the protein or fragment thereof, for example, an activator (*e.g.*, a trans-acting factor), a chaperone and a processing protease. The nucleic acids encoding one or more of these factors are preferably not operably linked to the nucleic acid encoding the protein or fragment thereof. An activator is a protein which activates transcription of a nucleic acid sequence encoding a polypeptide (Kudla *et al.*, *EMBO* 9:1355-1364(1990); Jarai and Buxton, *Current Genetics*



26:2238-244(1994); Verdier, *Yeast* 6:271-297(1990), all of which are herein incorporated by reference in their entirety). The nucleic acid sequence encoding an activator may be obtained from the genes encoding *Saccharomyces cerevisiae* heme activator protein 1 (hap1), *Saccharomyces cerevisiae* galactose metabolizing protein 4 (gal4) and *Aspergillus nidulans* ammonia regulation protein (areA). For further examples, see Verdier, *Yeast* 6:271-297 (1990); MacKenzie *et al.*, *Journal of Gen. Microbiol.* 139:2295-2307 (1993), both of which are herein incorporated by reference in their entirety). A chaperone is a protein which assists another protein in folding properly (Hartl *et al.*, *TIBS* 19:20-25 (1994); Bergeron *et al.*, *TIBS* 19:124-128 (1994); Demolder *et al.*, *J. Biotechnology* 32:179-189 (1994); Craig, *Science* 260:1902-1903(1993); Gething and Sambrook, *Nature* 355:33-45 (1992); Puig and Gilbert, *J Biol. Chem.* 269:7764-7771 (1994); Wang and Tsou, *FASEB Journal* 7:1515-11157 (1993); Robinson *et al.*, *Bio/Technology* 1:381-384 (1994), all of which are herein incorporated by reference in their entirety). The nucleic acid sequence encoding a chaperone may be obtained from the genes encoding *Aspergillus oryzae* protein disulphide isomerase, *Saccharomyces cerevisiae* calnexin, *Saccharomyces cerevisiae* BiP/GRP78 and *Saccharomyces cerevisiae* Hsp70. For further examples, see Gething and Sambrook, *Nature* 355:33-45 (1992); Hartl *et al.*, *TIBS* 19:20-25 (1994). A processing protease is a protease that cleaves a propeptide to generate a mature biochemically active polypeptide (Enderlin and Ogrydziak, *Yeast* 10:67-79 (1994); Fuller *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1434-1438 (1989); Julius *et al.*, *Cell* 37:1075-1089 (1984); Julius *et al.*, *Cell* 32:839-852 (1983), all of which are incorporated by reference in their entirety). The nucleic acid sequence encoding a processing protease may be obtained from the genes encoding *Aspergillus niger* Kex2, *Saccharomyces cerevisiae* dipeptidylaminopeptidase, *Saccharomyces cerevisiae* Kex2 and *Yarrowia lipolytica* dibasic processing endoprotease (xpr6). Any factor that is functional in the fungal host cell of choice may be used in the invention.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* host cells are described in EP 238 023 and

Yelton *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 81:1470-1474 (1984), both of which are herein incorporated by reference in their entirety. A suitable method of transforming *Fusarium* species is described by Malardier *et al.*, *Gene* 78:147-156 (1989), the entirety of which is herein incorporated by reference. Yeast may be transformed using the procedures described by Becker and Guarente, In: Abelson and Simon, (eds.), *Guide to Yeast Genetics and Molecular Biology, Methods Enzymol.* Volume 194, pp. 182-187, Academic Press, Inc., New York; Ito *et al.*, *J. Bacteriology* 153:163 (1983); Hinnen *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 75:1920 (1978), all of which are herein incorporated by reference in their entirety.

The invention also relates to methods of producing the protein or fragment thereof comprising culturing the recombinant fungal host cells under conditions conducive for expression of the protein or fragment thereof. The fungal cells of the invention are cultivated in a nutrient medium suitable for production of the protein or fragment thereof using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the protein or fragment thereof to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art (*see, e.g.*, Bennett and LaSure (eds.), *More Gene Manipulations in Fungi*, Academic Press, CA, (1991), the entirety of which is herein incorporated by reference). Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection, Manassas, VA). If the protein or fragment thereof is secreted into the nutrient medium, a protein or fragment thereof can be recovered directly from the medium. If the protein or fragment thereof is not secreted, it is recovered from cell lysates.

The expressed protein or fragment thereof may be detected using methods known in the art that are specific for the particular protein or fragment. These detection methods may include the use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme

substrate. For example, if the protein or fragment thereof has enzymatic activity, an enzyme assay may be used. Alternatively, if polyclonal or monoclonal antibodies specific to the protein or fragment thereof are available, immunoassays may be employed using the antibodies to the protein or fragment thereof. The techniques of enzyme assay and immunoassay are well known to those skilled in the art.

The resulting protein or fragment thereof may be recovered by methods known in the arts. For example, the protein or fragment thereof may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. The recovered protein or fragment thereof may then be further purified by a variety of chromatographic procedures, *e.g.*, ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like.

**(g) Mammalian Constructs and Transformed Mammalian Cells**

The invention also relates to methods for obtaining a recombinant mammalian host cell, comprising introducing into a mammalian host cell exogenous genetic material. The invention also relates to a mammalian cell comprising a mammalian recombinant vector. The invention also relates to methods for obtaining a recombinant mammalian host cell, comprising introducing into a mammalian cell exogenous genetic material. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC, Manassas, VA), such as HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells and a number of other cell lines.

Suitable promoters for mammalian cells are also known in the art and include viral promoters, such as those from Simian Virus 40 (SV40) (Fiers *et al.*, *Nature* 273: 113 (1978)), Rous sarcoma virus (RSV), adenovirus (ADV), cytomegalovirus (CMV), and bovine papilloma virus (BPV), as well as mammalian cell-derived promoters. An exemplary, non-limiting, list includes: a hematopoietic stem cell-specific promoter, such as the CD34 promoter (Burn *et al.*, U.S. Patent No. 5,556,954); the glucose-6-phosphatase promoter (Yoshiuchi *et al.*, *J. Clin. Endocrin. Metab.* 83:1016-1019 (1998)); interleukin-1 alpha promoter (Mori and Prager, *Leuk. Lymphoma* 26:421-433 (1997)); CMV promoter (Tong *et al.*, *Anticancer Res.* 18:719-725 (1998), Norman *et al.*, *Vaccine* 15:801-803 (1997)); RSV promoter (Elshami *et al.*, *Cancer Gene Ther.* 4:213-221 (1997); Baldwin *et al.*, *Gene Ther.* 4:1142-1149 (1997)); SV40 promoter (Harms and Splitter, *Hum. Gene Ther.* 6:1291-1297 (1995)); CD11c integrin gene promoter (Corbi and Lopez-Rodriguez, *Leuk. Lymphoma* 25:415-425 (1997)), GM-CSF promoter (Shannon *et al.*, *Crit. Rev. Immunol.* 17:301-323 (1997)); interleukin-5R alpha promoter (Sun *et al.*, *Curr. Top. Microbiol. Immunol.* 211:173-187 (1996)); interleukin-2 promoter (Serfing *et al.*, *Biochim. Biophys. Acta* 1263:181-200 (1995); O'Neill *et al.*, *Transplant Proc.* 23:2862-2866 (1991)); c-fos promoter (Janknecht, *Immunobiology* 193:137-142 (1995), Janknecht *et al.*, *Carcinogenesis* 16:443-450 (1995), Takai *et al.*, *Princess Takamatsu Symp.* 22:197-204 (1991)); h-ras promoter (Rachal *et al.*, *EXS* 64:330-342 (1993)); and DMD gene promoter (Ray *et al.*, *Adv. Exp. Med. Biol.* 280:107-111 (1990). All of the above documents are incorporated by reference in their entirety and can be relied on to make or use aspects of this invention, especially in designing and constructing appropriate vector and host expression systems.

Vectors used in mammalian cell expression systems may also include additional functional sequences. For example, terminator sequences, poly-A addition sequences, and internal ribosome entry site (IRES) sequences. Enhancer sequences, which increase expression, may also be included and sequences that promote amplification of the gene may also be desirable (for example, methotrexate resistance genes). One of skill in the art is familiar with numerous

examples of these additional functional sequences, as well as other functional sequences, that may optionally be included in an expression vector.

Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding HCV epitopes into the host genome. For example, another vector used to express foreign DNA is vaccinia virus. In this case, for example, a nucleic acid molecule encoding a protein or fragment thereof is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art and may utilize, for example, homologous recombination. Such heterologous DNA is generally inserted into a gene which is non-essential to the virus, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (*see*, for example, Mackett *et al*, *J Virol.* 49:857 (1984); Chakrabarti *et al.*, *Mol. Cell. Biol.* 5:3403 (1985); Moss, In: *Gene Transfer Vectors For Mammalian Cells* (Miller and Calos, eds., Cold Spring Harbor Laboratory, N.Y., p. 10, (1987); all of which are herein incorporated by reference in their entirety). Expression of the HCV polypeptide then occurs in cells or animals which are infected with the live recombinant vaccinia virus.

The sequence to be integrated into the mammalian sequence may be introduced into the primary host by any convenient means, which includes calcium precipitated DNA, spheroplast fusion, transformation, electroporation, biolistics, lipofection, microinjection, or other convenient means. Where an amplifiable gene is being employed, the amplifiable gene may serve as the selection marker for selecting hosts into which the amplifiable gene has been introduced. Alternatively, one may include with the amplifiable gene another marker, such as a drug resistance marker, *e.g.*, neomycin resistance (G418 in mammalian cells), hygromycin in resistance etc., or an auxotrophy marker (HIS3, TRP1, LEU2, URA3, ADE2, LYS2, etc.) for use in yeast cells.

Depending upon the nature of the modification and associated targeting construct, various techniques may be employed for identifying targeted integration. Conveniently, the DNA may be

digested with one or more restriction enzymes and the fragments probed with an appropriate DNA fragment which will identify the properly sized restriction fragment associated with integration.

One may use different promoter sequences, enhancer sequences, or other sequence which will allow for enhanced levels of expression in the expression host. Thus, one may combine an enhancer from one source, a promoter region from another source, a 5'- noncoding region upstream from the initiation methionine from the same or different source as the other sequences and the like. One may provide for an intron in the non-coding region with appropriate splice sites or for an alternative 3'- untranslated sequence or polyadenylation site. Depending upon the particular purpose of the modification, any of these sequences may be introduced, as desired.

Where selection is intended, the sequence to be integrated will have with it a marker gene, which allows for selection. The marker gene may conveniently be downstream from the target gene and may include resistance to a cytotoxic agent, *e.g.*, antibiotics, heavy metals, or the like, resistance or susceptibility to HAT, gancyclovir, etc., complementation to an auxotrophic host, particularly by using an auxotrophic yeast as the host for the subject manipulations, or the like. The marker gene may also be on a separate DNA molecule, particularly with primary mammalian cells. Alternatively, one may screen the various transformants, due to the high efficiency of recombination in yeast, by using hybridization analysis, PCR, sequencing, or the like.

For homologous recombination, constructs can be prepared where the amplifiable gene will be flanked, normally on both sides with DNA homologous with the DNA of the target region. Depending upon the nature of the integrating DNA and the purpose of the integration, the homologous DNA will generally be within 100kb, usually 50kb, preferably about 25kb, of the transcribed region of the target gene, more preferably within 2kb of the target gene. Where modeling of the gene is intended, homology will usually be present proximal to the site of the mutation. The homologous DNA may include the 5'-upstream region outside of the transcriptional regulatory region or comprising any enhancer sequences, transcriptional initiation

sequences, adjacent sequences, or the like. The homologous region may include a portion of the coding region, where the coding region may be comprised only of an open reading frame or combination of exons and introns. The homologous region may comprise all or a portion of an intron, where all or a portion of one or more exons may also be present. Alternatively, the homologous region may comprise the 3'-region, so as to comprise all or a portion of the transcriptional termination region, or the region 3' of this region. The homologous regions may extend over all or a portion of the target gene or be outside the target gene comprising all or a portion of the transcriptional regulatory regions and/or the structural gene.

The integrating constructs may be prepared in accordance with conventional ways, where sequences may be synthesized, isolated from natural sources, manipulated, cloned, ligated, subjected to in vitro mutagenesis, primer repair, or the like. At various stages, the joined sequences may be cloned and analyzed by restriction analysis, sequencing, or the like. Usually during the preparation of a construct where various fragments are joined, the fragments, intermediate constructs and constructs will be carried on a cloning vector comprising a replication system functional in a prokaryotic host, *e.g.*, *E. coli* and a marker for selection, *e.g.*, biocide resistance, complementation to an auxotrophic host, etc. Other functional sequences may also be present, such as polylinkers, for ease of introduction and excision of the construct or portions thereof, or the like. A large number of cloning vectors are available such as pBR322, the pUC series, etc. These constructs may then be used for integration into the primary mammalian host.

In the case of the primary mammalian host, a replicating vector may be used. Usually, such vector will have a viral replication system, such as SV40, bovine papilloma virus, adenovirus, or the like. The linear DNA sequence vector may also have a selectable marker for identifying transfected cells. Selectable markers include the neo gene, allowing for selection with G418, the herpes tk gene for selection with HAT medium, the gpt gene with mycophenolic acid, complementation of an auxotrophic host, etc.

The vector may or may not be capable of stable maintenance in the host. Where the vector is capable of stable maintenance, the cells will be screened for homologous integration of the vector into the genome of the host, where various techniques for curing the cells may be employed. Where the vector is not capable of stable maintenance, for example, where a temperature sensitive replication system is employed, one may change the temperature from the permissive temperature to the non-permissive temperature, so that the cells may be cured of the vector. In this case, only those cells having integration of the construct comprising the amplifiable gene and, when present, the selectable marker, will be able to survive selection.

Where a selectable marker is present, one may select for the presence of the targeting construct by means of the selectable marker. Where the selectable marker is not present, one may select for the presence of the construct by the amplifiable gene. For the neo gene or the herpes tk gene, one could employ a medium for growth of the transformants of about 0.1-1 mg/ml of G418 or may use HAT medium, respectively. Where DHFR is the amplifiable gene, the selective medium may include from about 0.01-0.5  $\mu$ M of methotrexate or be deficient in glycine-hypoxanthine-thymidine and have dialysed serum (GHT media).

The DNA can be introduced into the expression host by a variety of techniques that include calcium phosphate/DNA co-precipitates, microinjection of DNA into the nucleus, electroporation, yeast protoplast fusion with intact cells, transfection, polycations, *e.g.*, polybrene, polyornithine, etc., or the like. The DNA may be single or double stranded DNA, linear or circular. The various techniques for transforming mammalian cells are well known (see Keown *et al.*, *Methods Enzymol.* (1989); Keown *et al.*, *Methods Enzymol.* 185:527-537 (1990); Mansour *et al.*, *Nature* 336:348-352, (1988); all of which are herein incorporated by reference in their entirety).

#### **(h) Insect Constructs and Transformed Insect Cells**

The invention also relates to an insect recombinant vectors comprising exogenous genetic material. The invention also relates to an insect cell comprising an insect recombinant vector. The invention also relates to methods for obtaining a recombinant insect host cell, comprising



introducing into an insect cell exogenous genetic material. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

The insect recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleic acid sequence.

The choice of a vector will typically depend on the compatibility of the vector with the insect host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the insect host. In addition, the insect vector may be an expression vector. Nucleic acid molecules can be suitably inserted into a replication vector for expression in the insect cell under a suitable promoter for insect cells. Many vectors are available for this purpose and selection of the appropriate vector will depend mainly on the size of the nucleic acid molecule to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the particular host cell with which it is compatible. The vector components for insect cell transformation generally include, but are not limited to, one or more of the following: a signal sequence, origin of replication, one or more marker genes and an inducible promoter.

The insect vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the insect cell, is integrated into the genome and

replicated together with the chromosome(s) into which it has been integrated. For integration, the vector may rely on the nucleic acid sequence of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the insect host. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, there should be preferably two nucleic acid sequences which individually contain a sufficient number of nucleic acids, preferably 400bp to 1500bp, more preferably 800bp to 1000bp, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the insect host cell and, furthermore, may be non-encoding or encoding sequences.

Baculovirus expression vectors (BEVs) have become important tools for the expression of foreign genes, both for basic research and for the production of proteins with direct clinical applications in human and veterinary medicine (Doerfler, *Curr. Top. Microbiol. Immunol.* 131:51-68 (1968); Luckow and Summers, *Bio/Technology* 6:47-55 (1988a); Miller, *Annual Review of Microbiol.* 42:177-199 (1988); Summers, *Curr. Comm. Molecular Biology*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1988); all of which are herein incorporated by reference in their entirety). BEVs are recombinant insect viruses in which the coding sequence for a chosen foreign gene has been inserted behind a baculovirus promoter in place of the viral gene, *e.g.*, polyhedrin (Smith and Summers, U.S. Pat. No., 4,745,051, the entirety of which is incorporated herein by reference).

The use of baculovirus vectors relies upon the host cells being derived from *Lepidopteran* insects such as *Spodoptera frugiperda* or *Trichoplusia ni*. The preferred *Spodoptera frugiperda* cell line is the cell line Sf9. The *Spodoptera frugiperda* Sf9 cell line was obtained from American Type Culture Collection (Manassas, VA.) and is assigned accession number ATCC

CRL 1711 (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Ag. Exper. Station Bulletin No. 1555 (1988), the entirety of which is herein incorporated by reference). Other insect cell systems, such as the silkworm *B. mori* may also be used.

5           The proteins expressed by the BEVs are, therefore, synthesized, modified and transported in host cells derived from *Lepidopteran* insects. Most of the genes that have been inserted and produced in the baculovirus expression vector system have been derived from vertebrate species. Other baculovirus genes in addition to the polyhedrin promoter may be employed to advantage in a baculovirus expression system. These include immediate-early (alpha), delayed-early (β), late  
10 (γ), or very late (delta), according to the phase of the viral infection during which they are expressed. The expression of these genes occurs sequentially, probably as the result of a "cascade" mechanism of transcriptional regulation. (Guarino and Summers, *J. Virol.* 57:563-571 (1986); Guarino and Summers, *J. Virol.* 61:2091-2099 (1987); Guarino and Summers, *Virol.* 162:444-451 (1988); all of which are herein incorporated by reference in their entirety).

15           Insect recombinant vectors are useful as intermediates for the infection or transformation of insect cell systems. For example, an insect recombinant vector containing a nucleic acid molecule encoding a baculovirus transcriptional promoter followed downstream by an insect signal DNA sequence is capable of directing the secretion of the desired biologically active protein from the insect cell. The vector may utilize a baculovirus transcriptional promoter region  
20 derived from any of the over 500 baculoviruses generally infecting insects, such as for example the Orders *Lepidoptera*, *Diptera*, *Orthoptera*, *Coleoptera* and *Hymenoptera*, including for example but not limited to the viral DNAs of *Autographa californica* MNPV, *Bombyx mori* NPV, *Trichoplusia ni* MNPV, *Rachiplusia ou* MNPV or *Galleria mellonella* MNPV, wherein said baculovirus transcriptional promoter is a baculovirus immediate-early gene IEL or IEN promoter;  
25 an immediate-early gene in combination with a baculovirus delayed-early gene promoter region selected from the group consisting of 39K and a *HindIII-k* fragment delayed-early gene; or a baculovirus late gene promoter. The immediate-early or delayed-early promoters can be

enhanced with transcriptional enhancer elements. The insect signal DNA sequence may code for a signal peptide of a *Lepidopteran* adipokinetic hormone precursor or a signal peptide of the *Manduca sexta* adipokinetic hormone precursor (Summers, U.S. Patent No. 5,155,037; the entirety of which is herein incorporated by reference). Other insect signal DNA sequences include a signal peptide of the *Orthoptera Schistocerca gregaria* locust adipokinetic hormone precursor and the *Drosophila melanogaster* cuticle genes CP1, CP2, CP3 or CP4 or for an insect signal peptide having substantially a similar chemical composition and function (Summers, U.S. Patent No. 5,155,037).

Insect cells are distinctly different from animal cells. Insects have a unique life cycle and have distinct cellular properties such as the lack of intracellular plasminogen activators in which are present in vertebrate cells. Another difference is the high expression levels of protein products ranging from 1 to greater than 500 mg/liter and the ease at which cDNA can be cloned into cells (Frasier, *In Vitro Cell. Dev. Biol.* 25:225 (1989); Summers and Smith, In: *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Ag. Exper. Station Bulletin No. 1555 (1988), both of which are incorporated by reference in their entirety).

Recombinant protein expression in insect cells is achieved by viral infection or stable transformation. For viral infection, the desired gene is cloned into baculovirus at the site of the wild-type polyhedron gene (Webb and Summers, *Technique* 2:173 (1990); Bishop and Posse, *Adv. Gene Technol.* 1:55 (1990); both of which are incorporated by reference in their entirety).

The polyhedron gene is a component of a protein coat in occlusions which encapsulate virus particles. Deletion or insertion in the polyhedron gene results the failure to form occlusion bodies. Occlusion negative viruses are morphologically different from occlusion positive viruses and enable one skilled in the art to identify and purify recombinant viruses.

The vectors of invention preferably contain one or more selectable markers, which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides, for example biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs and the like. Selection may be accomplished by co-transformation, *e.g.*, as described in WO

91/17243, a nucleic acid sequence of the invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleic acid sequence, which is recognized by the insect host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences, which mediate the expression of the protein or fragment thereof. The promoter may be any nucleic acid sequence, which shows transcriptional activity in the insect host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell.

For example, a nucleic acid molecule encoding a protein or fragment thereof may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated region of a mRNA, which is important for translation by the fungal host. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the protein or fragment thereof. The leader sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence, which is functional in the insect host cell of choice may be used in the invention.

A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleic acid sequence of the invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the insect host to add polyadenosine residues to transcribed mRNA. The polyadenylation sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence, which is functional in the fungal host of choice, may be used in the invention.

To avoid the necessity of disrupting the cell to obtain the protein or fragment thereof and to minimize the amount of possible degradation of the expressed polypeptide within the cell, it is preferred that expression of the polypeptide gene gives rise to a product secreted outside the cell. To this end, the protein or fragment thereof of the invention may be linked to a signal peptide linked to the amino terminus of the protein or fragment thereof. A signal peptide is an amino acid sequence which permits the secretion of the protein or fragment thereof from the insect host into the culture medium. The signal peptide may be native to the protein or fragment thereof of

the invention or may be obtained from foreign sources. The 5' end of the coding sequence of the nucleic acid sequence of the invention may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted protein or fragment thereof.

5 At present, a mode of achieving secretion of a foreign gene product in insect cells is by way of the foreign gene's native signal peptide. Because the foreign genes are usually from non-insect organisms, their signal sequences may be poorly recognized by insect cells and, hence, levels of expression may be suboptimal. However, the efficiency of expression of foreign gene products seems to depend primarily on the characteristics of the foreign protein. On average, 10 nuclear localized or non-structural proteins are most highly expressed, secreted proteins are intermediate and integral membrane proteins are the least expressed. One factor generally affecting the efficiency of the production of foreign gene products in a heterologous host system is the presence of native signal sequences (also termed presequences, targeting signals, or leader sequences) associated with the foreign gene. The signal sequence is generally coded by a DNA 15 sequence immediately following (5' to 3') the translation start site of the desired foreign gene.

The expression dependence on the type of signal sequence associated with a gene product can be represented by the following example. If a foreign gene is inserted at a site downstream from the translational start site of the baculovirus polyhedrin gene so as to produce a fusion protein (containing the N-terminus of the polyhedrin structural gene), the fused gene is highly 20 expressed. But less expression is achieved when a foreign gene is inserted in a baculovirus expression vector immediately following the transcriptional start site and totally replacing the polyhedrin structural gene.

Insertions into the region -50 to -1 significantly alter (reduce) steady state transcription which, in turn, reduces translation of the foreign gene product. Use of the pVL941 vector 25 optimizes transcription of foreign genes to the level of the polyhedrin gene transcription. Even though the transcription of a foreign gene may be optimal, optimal translation may vary because

of several factors involving processing: signal peptide recognition, mRNA and ribosome binding, glycosylation, disulfide bond formation, sugar processing, oligomerization, for example.

The properties of the insect signal peptide are expected to be more optimal for the efficiency of the translation process in insect cells than those from vertebrate proteins. This phenomenon can generally be explained by the fact that proteins secreted from cells are synthesized as precursor molecules containing hydrophobic N-terminal signal peptides. The signal peptides direct transport of the select protein to its target membrane and are then cleaved by a peptidase on the membrane, such as the endoplasmic reticulum, when the protein passes through it.

Another exemplary insect signal sequence is the sequence encoding for *Drosophila* cuticle proteins such as CP1, CP2, CP3 or CP4 (Summers, U.S. Patent No. 5,278,050; the entirety of which is herein incorporated by reference). Most of a 9kb region of the *Drosophila* genome containing genes for the cuticle proteins has been sequenced. Four of the five cuticle genes contains a signal peptide coding sequence interrupted by a short intervening sequence (about 60 base pairs) at a conserved site. Conserved sequences occur in the 5' mRNA untranslated region, in the adjacent 35 base pairs of upstream flanking sequence and at -200 base pairs from the mRNA start position in each of the cuticle genes.

Standard methods of insect cell culture, cotransfection and preparation of plasmids are set forth in Summers and Smith (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experiment Station Bulletin No. 1555, Texas A&M University (1987)). Procedures for the cultivation of viruses and cells are described in Volkman and Summers, *J. Virol* 19:820-832 (1975) and Volkman *et al.*, *J. Virol* 19:820-832 (1976); both of which are herein incorporated by reference in their entirety.

#### (i) **Bacterial Constructs and Transformed Bacterial Cells**

The invention also relates to a bacterial recombinant vector comprising exogenous genetic material. The invention also relates to a bacteria cell comprising a bacterial recombinant vector. The invention also relates to methods for obtaining a recombinant bacteria host cell,

comprising introducing into a bacterial host cell exogenous genetic material. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention, preferably a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof or fragments of either. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

The bacterial recombinant vector may be any vector that can be conveniently subjected to recombinant DNA procedures. The choice of a vector will typically depend on the compatibility of the vector with the bacterial host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the bacterial host. In addition, the bacterial vector may be an expression vector. Nucleic acid molecules encoding protein homologues or fragments thereof can, for example, be suitably inserted into a replicable vector for expression in the bacterium under the control of a suitable promoter for bacteria. Many vectors are available for this purpose and selection of the appropriate vector will depend mainly on the size of the nucleic acid to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the particular host cell with which it is compatible. The vector components for bacterial transformation generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes and an inducible promoter.

In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with bacterial hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically



transformed using pBR322, a plasmid derived from an *E. coli* species (see, *e.g.*, Bolivar *et al.*, *Gene* 2:95 (1977); the entirety of which is herein incorporated by reference). The plasmid pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage,  
5 also generally contains, or is modified to contain, promoters that can be used by the microbial organism for expression of the selectable marker genes.

Nucleic acid molecules encoding protein or fragments thereof may be expressed not only directly, but also as a fusion with another polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature polypeptide. In  
10 general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide DNA that is inserted into the vector. The heterologous signal sequence selected should be one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For bacterial host cells that do not recognize and process the native polypeptide signal sequence, the signal sequence is substituted by a bacterial signal sequence selected, for example,  
15 from the group consisting of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and  
20 includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria.

Expression and cloning vectors also generally contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of  
25 transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin,

neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*. One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous protein homologue or fragment thereof produce a protein conferring drug resistance and thus survive the selection regimen.

The expression vector for producing a protein or fragment thereof can also contains an inducible promoter that is recognized by the host bacterial organism and is operably linked to the nucleic acid encoding, for example, the nucleic acid molecule encoding the protein homologue or fragment thereof of interest. Inducible promoters suitable for use with bacterial hosts include the  $\beta$ -lactamase and lactose promoter systems (Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281:544 (1979); both of which are herein incorporated by reference in their entirety), the arabinose promoter system (Guzman *et al.*, *J. Bacteriol.* 174:7716-7728 (1992); the entirety of which is herein incorporated by reference), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776; both of which are herein incorporated by reference in their entirety) and hybrid promoters such as the tac promoter (deBoer *et al.*, *Proc. Natl. Acad. Sci. (USA)* 80:21-25 (1983); the entirety of which is herein incorporated by reference). However, other known bacterial inducible promoters are suitable (Siebenlist *et al.*, *Cell* 20:269 (1980); the entirety of which is herein incorporated by reference).

Promoters for use in bacterial systems also generally contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the polypeptide of interest. The promoter can be removed from the bacterial source DNA by restriction enzyme digestion and inserted into the vector containing the desired DNA.

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored and re-ligated in the form desired to generate the plasmids required. Examples of available bacterial expression vectors include, but are not limited to: the multifunctional *E. coli* cloning and expression vectors such as Bluescript™ (Stratagene, La Jolla, CA), in which, for example,

encoding an *A. nidulans* protein homologue or fragment thereof homologue, may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke and Schuster, *J. Biol. Chem.* 264:5503-5509 (1989), the entirety of which is herein incorporated by reference);  
5 and the like. pGEX vectors (Promega, Madison Wisconsin U.S.A.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to optionally include a heparin, thrombin, or factor XA protease  
10 cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will. Proteins or polypeptides of the invention can be expressed as variants that facilitate purification. For example, a fusion protein to such proteins as maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX) are known in the art [New England BioLab, Beverly, Mass., Pharmacia, Piscataway, NJ, and InVitrogen, San Diego, CA]. The  
15 polypeptide or protein can also be a tagged variant to facilitate purification, such as with histidine or methionine rich regions (His-Tag; available from LifeTechnologies Inc, Gaithersburg, MD) that bind to metal ion affinity chromatography columns, or with an epitope that binds to a specific antibody (Flag, available from Kodak, New Haven, Conn.). An exemplary, non-limiting list of commercially available vectors suitable for fusion protein expression includes: pBR322  
20 (Promega); pGEX (Amersham); pT7 (USB); pET (Novagen); pIBI (IBI); pProEX-1 (Gibco/BRL); pBluescript II (Stratagene); pTZ18R and pTZ19R (USB); pSE420 (Invitrogen); pVL1392 (Invitrogen); pBlueBac (Invitrogen); pBacPAK (Clontech); pHIL (Invitrogen); pYES2 (Invitrogen); pCDNA (Invitrogen); and pREP (Invitrogen). A number of other purification methods or means are also known and can be used. Reverse-phase high performance liquid  
25 chromatography (RP-HPLC), optionally employing hydrophobic RP-HPLC media, *e.g.*, silica gel, further purify the protein. Combinations of methods and means can also be employed to provide a substantially purified recombinant polypeptide or protein. Isolated plasmids or DNA

fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required. Examples of available bacterial expression vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript Registered TM (Stratagene, La Jolla, CA), in which, for example, encoding an gene homologue or fragment thereof homologue, may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke and Schuster *J. Biol. Chem.* 264: 5503-5509 (1989). The entirety of which is herein incorporated by reference); and the like. pGEX vectors (Promega, Madison Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

Suitable host bacteria for a bacterial vector include archaebacteria and eubacteria, especially eubacteria and most preferably *Enterobacteriaceae*. Examples of useful bacteria include *Escherichia*, *Enterobacter*, *Azotobacter*, *Erwinia*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia*, *Shigella*, *Rhizobia*, *Vitreoscilla* and *Paracoccus*. Suitable *E. coli* hosts include *E. coli* W3110 (American Type Culture Collection (ATCC) 27,325, Manassas, Virginia U.S.A.), *E. coli* 294 (ATCC 31,446), *E. coli* B and *E. coli* X1776 (ATCC 31,537). These examples are illustrative rather than limiting. Mutant cells of any of the above-mentioned bacteria may also be employed. It is, of course, necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. *E. coli* strain W3110 is a preferred host or parent host because it is a common host strain for recombinant

DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes.

Host cells are transfected and preferably transformed with the above-described vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate and electroporation. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, (1989), is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO, as described in Chung and Miller (Chung and Miller, *Nucleic Acids Res.* 16:3580 (1988); the entirety of which is herein incorporated by reference). Yet another method is the use of the technique termed electroporation.

Bacterial cells used to produce the polypeptide of interest for purposes of this invention are cultured in suitable media in which the promoters for the nucleic acid encoding the heterologous polypeptide can be artificially induced as described generally, *e.g.*, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, (1989). Examples of suitable media are given in U.S. Pat. Nos. 5,304,472 and 5,342,763; both of which are incorporated by reference in their entirety.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (*e.g.*, DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989); Mailga *et al.*, *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995), the entirety of which

is herein incorporated by reference; Birren *et al.*, *Genome Analysis: Analyzing DNA*, 1, Cold Spring Harbor, New York, the entirety of which is herein incorporated by reference).

**(j) Algal Constructs and Algal Transformants**

The present invention also relates to an algal recombinant vector comprising exogenous genetic material. The present invention also relates to an algal cell comprising an algal recombinant vector. The present invention also relates to methods for obtaining a recombinant algal host cell comprising introducing into an algal host cell exogenous genetic material.

Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism. Exogenous genetic material may be transferred into an algal cell. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 621 or complements thereof. Another preferred class of exogenous genetic material are nucleic acid molecules that encode a protein having an amino acid selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragments thereof.

The algal recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures. The choice of a vector will typically depend on the compatibility of the vector with the algal host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the algal host.

The algal vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the algal cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. For integration, the vector

may rely on the nucleic acid sequence of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the algal host. The additional nucleic acid sequences enable the vector to be

5 integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, there should be preferably two nucleic acid sequences which individually contain a sufficient number of nucleic acids, preferably 400 bp to 1500 bp, more preferably 800 bp to 1000 bp, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. These

10 nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the algal host cell, and, furthermore, may be non-encoding or encoding sequences.

The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene, the product of which confers upon an algal cell resistance to a compound to which the algal would otherwise be

15 sensitive. The compound can be selected from the group consisting of antibiotics, fungicides, herbicides, and heavy metals. The selectable marker may be selected from any known or subsequently identified selectable markers, including markers derived from algal, fungal, and bacterial sources. Preferred selectable markers can be selected from the group including, but not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin

20 acetyltransferase), *ble* (bleomycin binding protein), *cat* (chloramphenicol acetyltransferase), *hygB* (hygromycin B phosphotransferase), *nat* (nourseothricin acetyltransferase), *niaD* (nitrate reductase), *neo* (neomycin phosphotransferase), *pac* (puromycin acetyltransferase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sat* (streptothricin acetyltransferase), *sC* (sulfate adenylyltransferase), *trpC* (anthranilate synthase), and glyphosate resistant EPSPS genes.

25 Furthermore, selection may be accomplished by co-transformation, *e.g.*, as described in WO 91/17243, herein incorporated by reference in its entirety.

A nucleic acid sequence of the present invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleic acid sequence which is recognized by the algal host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the protein or  
5 fragment thereof.

A promoter may be any nucleic acid sequence which shows transcriptional activity in the algal host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of a nucleic acid construct of the invention in an algal host are light harvesting  
10 protein promoters obtained from photosynthetic organisms, *Chlorella* virus methyltransferase promoters, CaMV 35 S promoter, PL promoter from bacteriophage  $\lambda$ , nopaline synthase promoter from the Ti plasmid of *Agrobacterium tumefaciens*, and bacterial trp promoter.

A protein or fragment thereof encoding nucleic acid molecule of the present invention may also be operably linked to a terminator sequence at its 3' terminus. The terminator sequence  
15 may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any terminator which is functional in the algal host cell of choice may be used in the present invention.

A protein or fragment thereof encoding nucleic acid molecule of the present invention may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated  
20 region of a mRNA which is important for translation by the algal host. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the protein or fragment thereof. The leader sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence which is functional in the algal host cell of choice may be used in the present invention.

A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleic acid sequence of the present invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the algal host to add polyadenosine residues to transcribed mRNA.  
25



The polyadenylation sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence which is functional in the algal host of choice may be used in the present invention.

The procedures used to ligate the elements described above to construct the recombinant expression vector of the present invention are well known to one skilled in the art (see, for example, Sambrook, 2nd ed., *et al.*, *Molecular Cloning, A Laboratory Manual Cold Spring Harbor, N.Y.*, (1989), herein incorporated by reference in its entirety).

The present invention also relates to recombinant algal host cells produced by the methods of the present invention which are advantageously used with the recombinant vector of the present invention. The cell is preferably transformed with a vector comprising a nucleic acid sequence of the invention followed by integration of the vector into the host chromosome. The choice of algal host cells will to a large extent depend upon the gene encoding the protein or fragment thereof and its source.

Algal cells may be transformed by a variety of known techniques, including but not limit to, microprojectile bombardment, protoplast fusion, electroporation, microinjection, and vigorous agitation in the presence of glass beads. Suitable procedures for transformation of green algal host cells are described in EP 108 580, herein incorporated by reference in its entirety. A suitable method of transforming *Chlorella* species is described by Jarvis and Brown, *Curr. Genet.* 19: 317-321 (1991), herein incorporated by reference in its entirety. A suitable method of transforming cells of diatom *Phaeodactylum tricornutum* species is described in WO 97/39106, herein incorporated by reference in its entirety. Chlorophyll C-containing algae may be transformed using the procedures described in US 5,661,017, herein incorporated by reference in its entirety.

The expressed protein or fragment thereof may be detected using methods known in the art that are specific for the particular protein or fragment. These detection methods may include the use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, if the protein or fragment thereof has enzymatic activity, an enzyme

assay may be used. Alternatively, if polyclonal or monoclonal antibodies specific to the protein or fragment thereof are available, immunoassays may be employed using the antibodies to the protein or fragment thereof. The techniques of enzyme assay and immunoassay are well known to those skilled in the art.

5           The resulting protein or fragment thereof may be recovered by methods known in the arts. For example, the protein or fragment thereof may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. The recovered protein or fragment thereof may then be further purified by a variety of chromatographic procedures, *e.g.*, ion exchange chromatography,  
10   gel filtration chromatography, affinity chromatography, or the like.

### **Computer Readable Media**

          The nucleotide or amino acid sequence provided in SEQ ID NO: 1 through SEQ ID NO: 626, or fragment thereof, or complement thereof, or a nucleotide or an amino acid sequence at least 70% identical, preferably 90% identical even more preferably 99% or about 100% identical  
15   to the sequence provided in SEQ ID NO: 1 through SEQ ID NO: 626, or where appropriate complement thereof or fragments of either, can be “provided” in a variety of mediums to facilitate use. Such a medium can also provide a subset thereof in a form that allows a skilled artisan to examine the sequences.

          A further preferred subset of nucleic acid sequences is where the subset of sequences is  
20   two proteins or fragments thereof, more preferably three proteins or fragments thereof and even more preferable four proteins or fragments thereof.

          In one application of this embodiment, a nucleotide sequence of the invention can be recorded on computer readable media so that a computer-readable medium comprises one or more of the nucleotide sequences of the invention. As used herein, “computer readable media”  
25   refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc, storage medium

and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

Any number of the sequences, or sequence fragments, of the nucleic acid molecules or proteins of the invention, or fragments of either, can be included, in any number of combinations,  
5 on a computer-readable medium. Specifically, any one or more of SEQ ID NO:1-626, or where appropriate, complements thereof, can be included.

A skilled artisan can readily appreciate how any computer readable medium can be used to create a machine or method comprising a computer readable medium having recorded thereon a nucleotide sequence of the invention. As used herein, "recorded" refers to a process for storing  
10 information on computer readable medium. A skilled artisan can readily adopt any method for recording information on computer readable medium to generate media comprising the nucleotide sequence information of the invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the invention. The choice of the data storage structure will generally be  
15 based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect or Microsoft Word, or represented in the form of an ASCII file, stored in a database application,  
20 such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the invention.

By providing one or more of nucleotide sequences of the invention, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is  
25 publicly available that allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), the entirety of which

is herein incorporated by reference) and BLAZE (Brutlag *et al.*, *Comp. Chem.* 17:203-207 (1993), the entirety of which is herein incorporated by reference) search algorithms on a Sybase system can be used to identify open reading frames (ORFs) within the genome that contain homology to ORFs or proteins from other organisms. Such ORFs are protein-encoding fragments within the sequences of the invention and are useful in producing commercially important proteins such as enzymes used in amino acid biosynthesis, metabolism, transcription, translation, RNA processing, nucleic acid and a protein degradation, protein modification and DNA replication, restriction, modification, recombination and repair.

The invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the nucleic acid molecule of the invention. As used herein, “a computer-based system” refers to the hardware means, software means and data storage means used to analyze the nucleotide sequence information of the invention. The minimum hardware means of the computer-based systems of the invention comprises a central processing unit (CPU), input means, output means and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the invention.

As indicated above, the computer-based systems of the invention comprise a data storage means having stored therein a nucleotide sequence of the invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, “data storage means” refers to memory that can store nucleotide sequence information of the invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the invention. As used herein, “search means” refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequence of the invention that match a particular target sequence or target motif. A variety of known algorithms are

disclosed publicly and a variety of commercially available software for conducting search means are available can be used in the computer-based systems of the invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTIN and BLASTIX (NCBIA). One of the available algorithms or implementing software packages for conducting  
5 homology searches can be adapted for use in the present computer-based systems.

The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that during searches for commercially important fragments of the nucleic acid molecules of the invention, such as sequence fragments involved in gene expression and protein processing, may be of  
10 shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequences the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are  
15 not limited to, enzymatic active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, *cis* elements, hairpin structures and inducible expression elements (protein binding sequences).

Thus, the invention further provides an input means for receiving a target sequence, a data storage means for storing the target sequences of the invention sequence identified using a  
20 search means as described above and an output means for outputting the identified homologous sequences. A variety of structural formats for the input and output means can be used to input and output information in the computer-based systems of the invention. A preferred format for an output means ranks fragments of the sequence of the invention by varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan  
25 with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments sequence of the invention. For example, implementing software which implement the BLAST and BLAZE algorithms (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) can be used to identify open frames within the nucleic acid molecules of the invention. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the invention.

Having now described the invention, the following examples are provided by way of illustration and are not intended to limit the scope of the invention, unless specified.

### Example 1

#### Identification of Yeast HES1

The yeast strain LPY9 (MATa, leu2, Ura3, his3) is grown overnight and inoculated into SD+ hul (histidine, uracil, leucine) media. Aliquots of the culture are treated with ketoconazole (an inhibitor of C-14 $\alpha$  demethylase (P450<sub>14DM</sub>) enzyme) at 10ug/ml, 50ug/ml, and 100ug/ml, corresponding to 10ppm, 50ppm, and 100ppm, respectively. A sample of each is collected at 2, 4, and 6 hours after treatment. Control samples treated with DMSO (dimethyl sulfoxide-solvent for ketoconazole) but not with ketoconazole are also collected. Total RNA from each sample is collected by conventional methods, such as a Zirconium/Silica bead binding and extraction method. The sequence content of each sample is analyzed and compared by hybridizing each of them to a number of yeast ORF sequences immobilized on a Nylon membrane in an array format.

A similar comparison of a wild type yeast strain and a double mutant strain is made. The double mutant CJ517 (MATa, erg11::URA3, erg3::LEU2, leu2, ura3, his4) [erg11, erg3 double mutant] is compared to LPY9 after growth in both YPD and SD+hul media. Samples are collected at approximately 0, 2, 4, and 6 hours after inoculation.

Table 2, below, lists the RNAs in each sample whose abundance is effected by ketoconazole treatment or whose abundance differs between wild type and the double mutant strain. The table also lists the corresponding gene or sequence identifier for those RNAs. The RNAs are ranked by the ratio of either ketocanozole vs. control or mutant vs. control, using the ratio of 50ppm ketocanozole/control as a basis for comparison.

**Table 2\***

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
30	YOR237W	(HES1)	134.648161	1417.6262	1358.1235	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins
31	YKL198C	(PTK1)	68.5845326	111.1984	233.11762	Serine/threonine protein kinase, activator of low-affinity, low-capacity polyamine transport
32	YLR465C	-	97.9601498	104.52215	133.57826	Protein of unknown function, questionable ORF
33	YMR129W	(POM152)	5.10206225	82.813831	15.392788	Nuclear pore membrane glycoprotein, type II integral membrane protein with N-terminal region on pore side and C-terminal region in the cisternae
34	YBR284W	-	4.92774291	60.027955	8.5359554	Protein with similarity to AMP deaminase
35	YKL158W	-	11.6717854	59.827307	75.220412	Protein of unknown function
36	YOR083W	-	31.7378598	51.606081	42.301568	Protein of unknown function
37	YOL095C	-	3.60507866	49.740211	21.834188	Protein with similarity to S. aureus DNA helicase PCRA
38	YOR188W	(MSB1)	2.19997209	42.446767	61.303817	Protein that may play a role in polarity establishment and bud formation
39	YBL109W	-	0.08616121	38.653463	75.964757	-
40	YLR091W	-	17.5946744	38.325073	44.556481	Protein of unknown function
41	YNL106C	(INP52)	2.52986454	35.205536	17.376557	-
42	YDR213W	-	18.2079478	32.136065	58.358612	Protein with similarity to transcription factors, has ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
43	YBL004W	-	8.49387973	28.614573	28.645633	Protein with similarity to members of the major facilitator superfamily (MFS)
44	YIR019C	(MUC1)	48.7538739	27.594853	137.77885	Glucoamylase I (alpha-1,4-glucan glucosidase), extracellular enzyme
45	YJL182C	-	2.53469593	26.891434	29.499298	Protein of unknown function

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
46	YMR254C	-	0.19897977	26.633459	10.625738	Protein of unknown function, questionable ORF
47	YDL134C	(PPH21)	3.51284473	22.849241	0	-
48	YCR098C	(GIT1)	2.27672091	21.746838	24.724171	Protein involved in inositol metabolism
49	YPL150W	-	4.72964069	21.633895	34.40982	Serine/threonine protein kinase of unknown function
50	YKL110C	(KTI12)	19.7752946	21.085633	16.303432	Protein involved in resistance to kluyveromyces lactis killer toxin
51	YER011W	(TIR1)	31.4723195	20.454605	17.935906	Stress-induced cell wall structural protein of the PAU1 family
52	YDL024C	-	3.96163383	20.381493	30.488098	Protein with similarity to acid phosphatases
53	YGR013W	-	0.10491681	20.364081	0	-
54	YOR325W	-	47.3518002	20.211317	29.305064	Protein of unknown function
55	YJR150C	-	159.265973	19.793221	13.560079	-
56	YDL126C	(CDC48)	42.7590386	19.0472	15.014024	Protein of the AAA family of ATPases, required for cell division and homotypic membrane fusion
57	YLR464W	-	12.4297115	18.580843	36.516503	Protein with similarity to other subtelomerically-coded proteins
58	YLR124W	-	0.13902212	18.351487	11.026125	Protein of unknown function
59	YLR463C	-	8.49721471	18.007814	29.811632	Protein with similarity to other subtelomerically-coded proteins
60	YMR297W	(PRC1)	6.20117404	17.995865	24.291751	Carboxypeptidase Y (CPY) (YSCY), serine-type protease
61	YFL029C	(CAK1)	17.1104765	16.96782	44.352291	CDK-activating kinase (serine/threonine protein kinase) responsible for in vivo activation of CDC28P, also involved in spore wall formation
62	YER054C	(GIP2)	2.14214491	16.442373	15.284537	GLC7P-interacting protein, possible regulatory subunit for the PP1 family protein phosphatase GLC7P
63	YER060W-A	(FCY22)	2.61677424	15.768882	20.550953	Purine-cytosine permease with similarity to FCY2P, member of the purine/cytosine family of the major facilitator superfamily (MFS)
64	YEL076C	-	13.7918147	14.372278	26.325282	Protein with similarity to other subtelomerically-encoded proteins
65	YGL176C	-	9.0823019	14.17085	16.23816	Protein with similarity to discopyge OMMATA CA++ channel alpha1 subunit protein B47447
66	YNR005C	-	12.9230524	14.032659	13.011356	Protein of unknown function, questionable ORF
67	YML032C-A	-	6.92372404	13.847081	5.501802	-
68	YGR190C	-	22.9885796	13.701633	42.22779	Protein of unknown function
69	YHR213W	-	17.3140804	13.267403	21.010074	Protein with similarity to the N-terminus of FLO1P and identical to YAR062P, probable pseudogene



Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
70	YPL272C	-	24.778114	12.93877	11.647985	Protein of unknown function
71	YBL100C	-	4.8456884	12.432421	16.193059	Protein of unknown function
72	YLR024C	-	11.2130442	11.927798	17.73046	Protein with similarity to ubiquitin-protein ligase (E3) UBR1P
73	YMR102C	-	4.61311719	11.865115	16.370862	-
74	YGR177C	(ATF2)	3.7081426	11.830167	12.555269	Alcohol O-acetyltransferase
75	YFR034C	(PHO4)	14.8112083	11.216073	20.844515	Basic helix-loop-helix (BHLH) transcription factor required for expression of phosphate pathway, hyperphosphorylation by PHO80P-PHO85P cyclin-dependent protein kinase complex causes inactivation
76	YNL282W	-	5.01708646	10.943286	13.050614	-
77	YPL176C	-	7.30789994	10.664169	18.424583	Protein with similarity to SSP134P
78	YMR015C	(ERG5)	10.2651358	10.313689	9.3557963	Cytochrome P450 (C-22 sterol desaturase)
79	YCR061W	-	4.07462743	10.291287	12.602668	Protein of unknown function
80	YHL030W	(ECM29)	4.85453872	10.275837	8.9818305	Protein possibly involved in cell wall structure or biosynthesis
81	YPL036W	(PMA2)	7.19300398	10.171951	12.917306	H <sup>+</sup> -transporting P-type ATPase of the plasma membrane, expression not detected under normal growth conditions
82	YFR007W	-	2.58144987	10.102403	6.0105766	Protein of unknown function
83	YOL067C	(RTG1)	30.4142081	10.027065	27.36633	Basic helix-loop-helix (BHLH) transcription factor involved in inter-organelle communication between mitochondria, peroxisomes, and nucleus
84	YGR265W	-	22.156977	9.9554618	5.672919	Protein of unknown function
85	YGR293C	-	51.4998515	9.7686634	8.066486	Protein of unknown function
86	YMR008C	(PLB1)	5.68517668	9.602215	11.309345	Phospholipase B (lysophospholipase), releases fatty acids from lysophospholipids
87	YOR140W	-	6.33829162	9.2015298	12.881145	-
88	YML034W	-	4.44092944	9.2011248	15.848216	Protein of unknown function
89	YGR176W	-	4.56487981	8.8866015	12.598661	Protein of unknown function
90	YOR014W	(RTS1)	7.03478812	8.8422619	11.590438	Protein serine/threonine phosphatase 2A (PP2A), B' regulatory subunit, involved in regulation of stress-related responses and the cell cycle
91	YMR317W	-	25.9636363	8.6834125	11.973301	Protein of unknown function
92	YOR301W	-	11.3702021	8.6327901	13.589223	Protein of unknown function
93	YER119C-A	-	8.9509545	8.4086333	6.8517264	-
94	YOR385W	-	6.30021483	8.3714543	10.537348	Protein of unknown function
95	YGL156W	(AMS1)	11.9450551	8.2732125	9.9190578	Alpha-mannosidase, hydrolyzes terminal non-reducing alpha-D-mannose residues from alpha-D-mannosides
96	YJL219W	(HXT9)	6.10093958	8.1969449	14.860533	Hexose transporter, member of the sugar permease family

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
97	YFL053W	-	3.55404282	8.1217569	6.04425	-
98	YNL279W	-	2.75618909	8.0041323	12.470971	Protein of unknown function
99	YHR007C	(ERG11)	5.511691	7.8623796	8.6320676	Cytochrome P450 (lanosterol 14alpha-demethylase), essential for biosynthesis of ergosterol
100	YJL127C	(SPT10)	4.01528284	7.8394427	10.096027	Protein that amplifies the magnitude of transcriptional regulation at various loci
101	YPL044C	-	2.61973879	7.8291062	4.5399013	Protein of unknown function
102	YOR030W	(DFG16)	4.97362211	7.8182123	10.573213	Protein involved in invasive growth upon nitrogen starvation
103	YIL011W	-	4.59710634	7.3954743	6.7112038	Protein with similarity to YIL176P, YIR041P and other members of the PAU1 family
104	YNR069C	-	14.3161508	7.3694614	14.104044	Protein of unknown function
105	YNL083W	-	2.06305137	7.3050052	15.674556	Protein of the mitochondrial carrier (MCF) family
106	YJL020C	-	6.76775321	7.0352757	5.3432583	Protein of unknown function
107	YFL065C	-	13.5712126	7.0075571	16.704839	Protein with similarity to other subtelomerically-encoded proteins including YHL049P, YIL177P, YJL225P, YER190P, YHR218P, and YEL076P
108	YNL329C	(PAS8)	3.75487269	6.7699941	25.980939	-
109	YHR006W	(STP2)	6.44648003	6.5480808	9.270283	Protein involved in tRNA splicing and branched-chain amino acid uptake
110	YJL221C	(FSP2)	2.37104879	6.4365653	6.3055084	Protein with similarity to alpha-D-glucosidase (maltase) (FSP2 and YIL172C code for identical proteins) (YIL172C and YGR287C are nearly identical)
111	YMR037C	(MSN2)	6.80686734	6.4235969	7.6612989	Zinc-finger transcriptional activator for genes involved in the multistress response and genes regulated through SNF1P
112	YLR379W	-	6.34038543	6.4227358	6.8206953	Protein of unknown function
113	YLR056W	(ERG3)	0.03858406	6.2735601	5.191422	C-5 sterol desaturase, an iron non-heme oxygen-requiring enzyme of the ergosterol biosynthesis pathway
114	YMR319C	(FET4)	3.5515443	6.2641804	8.194608	Low-affinity Fe(II) transport protein
115	YBR045C	(GIP1)	5.88011982	6.254107	3.8135044	GLC7P-interacting protein, possible regulatory subunit for the PP1 family protein phosphatases GLC7P
116	YKL147C	-	4.54862611	6.2431328	10.034699	Protein of unknown function
117	YMR135W-A	-	15.3287997	6.1049555	4.611173	-
118	YCR048W	(ARE1)	9.11370518	6.1039374	10.531291	Acyl-CoA:sterol acyltransferase (sterol-ester synthetase)

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
119	YBR235W	-	2.65851474	6.1026186	2.9854465	Protein with similarity to human SLC12A1 gene for which mutations are the cause of Bartter's Syndrome
120	YJL160C	-	5.14571281	6.0795621	6.0193217	Protein with similarity to members of the PIR1P/HSP150P/PIR3P family
121	YNL287W	(SEC21)	5.55890054	6.0742978	5.8985117	Coatomer complex gamma chain (gamma-COP) of secretory pathway vesicles, required for retrograde Golgi to endoplasmic reticulum transport
122	YLR458W	-	28.2501296	5.9435623	4.6311951	-
123	YLR121C	-	4.04284936	5.9154936	8.131848	-
124	YLR347C	(KAP95)	3.84797845	5.8759152	6.4154978	Karyopherin-beta, acts to target proteins with nuclear localization (NLS) sequences to the nuclear pore complex
125	YDL023C	-	3.26329833	5.8589624	4.7058193	Protein of unknown function
126	YAL010C	(MDM10)	5.34077952	5.807758	8.9195451	Protein involved in mitochondrial morphology and inheritance, mutant has large spherical mitochondria that do not move into the bud
127	YDR077W	(SED1)	3.30340602	5.6959082	5.9206909	Abundant cell surface glycoprotein, overexpression suppresses growth defect of ERD2
128	YDR247W	-	3.28497642	5.6793015	6.7651448	Serine/threonine protein kinase with similarity to S. pombe RAN1 negative regulator of sexual conjugation and meiosis (GB:Z49701)
129	YBL011W	-	3.59243122	5.650363	8.393684	-
130	YDL025C	-	2.91426204	5.5604876	3.9241843	Serine/threonine protein kinase with similarity to members of the NPR1 subfamily
131	YAL013W	(DEP1)	8.79366086	5.5463386	6.42501	Regulator of phospholipid metabolism
132	YIL084C	(SDS3)	1.99582364	5.5430688	6.9074225	Suppressor of silencing defect
133	YJL213W	-	7.09632444	5.4980741	5.5079382	Protein with weak similarity to nocardia arylalcoholphosphatase
134	YKR053C	-	5.37724431	5.4952302	6.4562635	-
135	YNR042W	-	17.7115615	5.4798109	7.5527661	Protein of unknown function
136	YCR072C	-	5.34712592	5.4565375	4.5985045	Protein with similarity to nuclear mRNA processing protein PRP4P, member of WD (WD-40) repeat family
137	YER086W	(ILV1)	4.55278717	5.4449008	4.2437712	Serine and threonine dehydratase (anabolic), first step in isoleucine biosynthesis pathway
138	YJL076W	-	11.4128793	5.4277219	6.6898119	-
139	YLR072W	-	5.19287856	5.4152299	7.2827024	Protein of unknown function

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
140	YDR301W	(YHH1)	2.51614995	5.4121298	7.0975432	-
141	YIL055C	-	2.0005314	5.3410327	4.6542324	Protein of unknown function
142	YEL076W-C	-	13.2032684	5.3265661	8.0731692	-
143	YNR047W	-	4.44731559	5.3217828	6.1790659	Serine/threonine protein kinase of unknown function
144	YGL211W	-	4.00934024	5.2957602	5.5379668	Protein of unknown function
145	YGL012W	(ERG4)	4.57738431	5.2945042	4.833773	Sterol C-24 reductase
146	YCL014W	(BUD3)	2.0970839	5.2855114	3.3963317	Protein localized at the neck filament ring required for axial budding, may provide a memory of the previous bud site
147	YBR106W	-	5.74228482	5.2537051	9.2061479	-
148	YHR095W	-	5.25923706	5.2434619	2.2666062	Protein of unknown function
149	YEL010W	-	3.39547744	5.2424909	3.9026395	Protein of unknown function
150	YBR005W	-	5.58242328	5.2283592	7.5591013	Protein of unknown function
151	YPL183C	-	3.25331232	5.2150911	4.034456	Protein of unknown function, has WD (WD-40) repeats
152	YJL159W	-	5.95901062	5.2095163	5.0420867	-
153	YBL065W	-	5.04084137	5.1918263	10.287249	Protein of unknown function
154	YDL071C	-	7.24874297	5.1844239	7.5184825	Protein of unknown function
155	YGR197C	(SNG1)	6.43784806	5.17339	7.8870948	Probable transport protein that confers resistance to MNNG and nitrosoguanidine
156	YLL028W	-	9.27382002	5.0519624	5.3421753	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
157	YKR034W	(DAL80)	3.91750209	5.0436172	7.2838566	GATA-type zinc finger transcriptional repressor for allantoin and 4-aminobutyric acid (GABA) catabolic genes
158	YDR430C	-	2.19022255	5.0401778	3.1989703	Protein with similarity to Class I family of aminoacyl-TRNA synthetases
159	YPL274W	-	5.4156341	5.0164198	6.1307085	Protein with similarity to GAP1P and other amino acid permeases
160	YMR261C	(TPS3)	3.96385669	4.94376	3.7501015	Component of the trehalose-6-phosphate synthase/phosphatase complex, alternate third subunit with TLS1P
161	YOL118C	-	3.20265396	4.936553	5.7544219	Protein of unknown function
162	YOR005C	(DNL4)	4.47086248	4.8815521	3.6707508	ATP-dependent DNA ligase IV, involved in non-homologous DNA end joining
163	YNL332W	-	3.33896215	4.8789948	4.7570682	-
164	YDR069C	(DOA4)	3.37810593	4.8769723	5.1947947	Ubiquitin-specific protease (ubiquitin C-terminal hydrolase) of the 26S proteasome complex, involved in vacuole biogenesis and osmoregulation
165	YOR009W	-	59.4543494	4.8708102	5.2948993	Protein with similarity to members of the PAU1 family

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166	YMR035W	(IMP2)	9.23409301	4.8492871	5.7664813	Inner membrane protease of mitochondria, acts in complex with IMP1P but has different substrate specificity for removal of signal peptidase
167	YER089C	(PTC2)	2.23920866	4.8455014	5.8687657	Protein serine/threonine phosphatase of the PP2C family
168	YJR018W	-	5.54754057	4.8389334	4.4934937	Protein of unknown function
169	YLR088W	(GAA1)	3.1893544	4.814116	4.0142997	Protein required for attachment of GPI anchor onto proteins, affects endocytosis
170	YOL163W	-	3.92239312	4.8014959	4.5124682	Protein with weak similarity to pseudomonas putida phthalate transporter
171	YLR462W	-	3.32915042	4.7928645	7.1350658	Protein of unknown function
172	YLR098C	(CHA4)	2.05280928	4.7564347	5.5866465	Zinc-finger protein required for activation of CHA1, has A ZN[2]-CYS[6] fungal-type binuclear cluster domain
173	YNR053C	-	2.55991235	4.7234659	3.8186389	Protein with similarity to human breast tumor-associated autoantigen
174	YDL246C	-	2.43826188	4.6757263	3.5757353	Protein with similarity to SOR1P (SOR1 and YDL246C code for nearly identical proteins)
175	YOL045W	-	3.55662236	4.672513	2.0538279	Serine/threonine protein kinase of unknown function
176	YKL176C	-	3.32695888	4.6429893	5.4538239	Protein of unknown function
177	YJR114W	-	3.00664482	4.6389866	4.0045917	Protein of unknown function
178	YER091C	(MET6)	6.67067887	4.6224571	2.9292597	Homocysteine methyltransferase (5-methyltetrahydropteroyl triglutamate--homocysteine methyltransferase), methionine synthase, cobalamin-independent
179	YHL049C	-	5.15537247	4.5637645	9.5066446	Protein with similarity to other subtelomerically-encoded proteins including YER189P, YML133P, and YJL225P, coded from a subtelomeric Y' region
180	YDR389W	(SAC7)	3.89197011	4.5609599	4.3143109	GTPase-activating protein for RHO1P
181	YMR202W	(ERG2)	9.58572292	4.5446614	5.575174	Sterol C8-C7 isomerase (C-8 sterol isomerase), enzyme of the ergosterol biosynthesis pathway
182	YBL019W	-	3.45990928	4.4694518	4.1655454	-
183	YGR287C	-	10.2933872	4.4595137	9.7718104	Protein with similarity to alpha-D-glucosidase (maltase) (YGR287C IS nearly identical to FSP2 and YIL172C)
184	YJL082W	-	7.42175571	4.4522595	5.556901	Protein of unknown function
185	YHR098C	-	2.51284975	4.4353768	4.3716652	Protein of unknown function
186	YOR371C	-	2.47743776	4.4289864	5.2783501	Protein of unknown function

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
187	YDR530C	(APA2)	2.40849553	4.3993312	2.7389073	ATP adenyllyltransferase II (AP4A phosphorylase)
188	YKL119C	(VPH2)	0.16462534	4.3613346	0	Vacuolar H-ATPase (V-ATPase) assembly protein acting in the endoplasmic reticulum
189	YOR273C	-	13.0544715	4.3469302	10.649131	Protein with similarity to members of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
190	YPL042C	(SSN3)	6.78272968	4.3344728	3.9578568	Cyclin-dependent serine/threonine protein kinase of the RNA polymerase II holoenzyme complex and Kornberg's mediator (SRB) subcomplex
191	YGR268C	-	4.77373538	4.3329069	5.2744105	Protein of unknown function
192	YPR011C	-	2.0077462	4.3123349	4.2742986	Protein with similarity to human Grave's Disease carrier protein (SP:P16260) and to bovine homolog of Grave's Disease carrier protein (SP:Q01888)
193	YPL022W	(RAD1)	4.48327554	4.3036056	6.5285426	Component of the nucleotide excision repairsome, homolog of human XPF xeroderma pigmentosum gene product and the mammalian ERCC-4 protein
194	YGL207W	(SPT16)	5.34289635	4.3033021	3.5727713	General chromatin factor required for adequate expression of CLN and other genes
195	YGL167C	(PMR1)	4.12359747	4.2628564	4.8141347	CA+++-transporting P-type ATPase of Golgi membrane involved in CA++ import into Golgi
196	YJR091C	(JSN1)	4.56429439	4.2419881	4.7804157	Protein that when overexpressed can suppress the hyperstable microtubule phenotype of TUB2-150
197	YDR238C	(SEC26)	4.48641405	4.2179222	3.8109695	Coatomer complex beta chain (beta-COP) of secretory pathway vesicles, required for retrograde transport from Golgi to endoplasmic reticulum
198	YDL012C	-	2.90930997	4.2158147	2.0519798	Protein of unknown function
199	YDR044W	(HEM13)	14.9283272	4.2136787	3.4946018	Coproporphyrinogen III oxidase, oxygen-repressed, sixth step in heme-biosynthetic pathway
200	YGL114W	-	3.22707938	4.2023503	5.0073787	Protein with similarity to S. pombe ISP4 protein, member of the major facilitator superfamily (MFS)
201	YGL055W	(OLE1)	2.29875509	4.1923045	3.5992372	Stearoyl-CoA desaturase (delta-9 fatty acid desaturase), required for synthesis of unsaturated fatty acids

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202	YDL088C	(ASM4)	4.39685251	4.1757265	3.321034	Suppressor of temperature-sensitive mutations in POL3P (DNA polymerase delta)
203	YKL171W	-	2.64137608	4.1581147	8.2933538	Serine/threonine protein kinase of unknown function
204	YPL190C	-	5.94196213	4.1575162	3.202837	-
205	YMR140W	-	5.24432896	4.157179	5.4545409	Protein of unknown function
206	YBL005W	(PDR3)	3.75060207	4.1449054	6.0827305	Transcription factor related to PDR1P, contains a ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
207	YML032C	(RAD52)	3.13968668	4.1330793	3.0832115	Protein required for recombination and repair of X-ray damage, has a late function in meiotic recombination
208	YFR018C	-	5.28886874	4.1041589	6.4001917	Protein with similarity to human glutamyl-peptide cyclotransferase
209	YGL125W	(MET11)	6.80542292	4.0762178	4.4382484	-
210	YCR057C	(PWP2)	3.34704165	4.0555292	3.4118145	-
211	YBL044W	-	4.67885642	4.0526493	9.1998322	Protein of unknown function
212	YPL268W	(PLC1)	2.90633764	4.0372127	2.1993847	Phosphoinositide-specific phospholipase C (1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase 1), produces diacylglycerol and inositol 1,4,5-trisphosphate
213	YOR204W	(DED1)	2.52920945	4.0291663	3.0830731	ATP-dependent RNA helicase of dead box family involved in protein synthesis
214	YPL171C	(OYE3)	4.94122983	4.0225239	0.2747214	NAPDH dehydrogenase (old yellow enzyme), isoform 3
215	YOR203W	-	3.473713	3.9900922	3.2232019	Protein of unknown function
216	YNL295W	-	2.855052	3.9889367	2.1389666	Protein of unknown function
217	YEL042W	(GDA1)	2.00067395	3.9856733	3.8058139	Guanosine diphosphatase of Golgi membrane
218	YLR339C	-	4.12619065	3.9844972	3.506939	Protein of unknown function
219	YIL007C	-	5.11957134	3.9770005	2.318674	Protein of unknown function
220	YIR007W	-	3.71972069	3.9670484	5.0861072	Protein with similarity to endoglucanase
221	YER114C	(BOI2)	2.5967273	3.9643546	6.3042836	Protein with SH3 domain involved in bud formation, binds to BEM1P
222	YLR092W	(SUL2)	6.02237117	3.9547891	4.5438793	High-affinity sulfate transporter
223	YEL060C	(PRB1)	5.60961951	3.939317	4.7370327	Protease B (YSCB) (PRB) (cerevisin), serine protease of the subtilisin family with broad proteolytic specificity
224	YAL051W	-	2.40553928	3.9334781	4.5099518	-
225	YJR147W	-	2.05911726	3.9267937	5.1956856	-
226	YOR386W	(PHR1)	1.99823774	3.9204076	5.7096569	Deoxyribodipyrimidine photolyase, involved in light-dependent repair of pyrimidine dimers

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
227	YCR037C	(PHO87)	2.81200613	3.8982171	4.0311884	Member of the phosphate permease family of the major facilitator superfamily
228	YOL100W	-	3.04632994	3.8854218	9.2592192	Serine/threonine protein kinase of unknown function
229	YBL047C	-	3.40597241	3.88363	5.2814809	Protein with similarity to cytoskeletal protein USO1P, PAN1P, and mouse tyrosine kinase substrate EPS15
230	YAR014C	-	2.53512963	3.823947	4.4065791	Protein of unknown function
231	YKL182W	(FAS1)	3.75368336	3.8068781	5.3493259	Fatty-acyl-CoA synthase, beta chain (contains acetyl transferase, enoyl reductase, dehydratase, and malonyl/palmitoyl transferase)
232	YLR331C	-	3.94099666	3.795387	3.3715843	Protein of unknown function
233	YEL031W	(SPF1)	7.77512435	3.7891074	4.357615	Protein with similarity to CA++-transporting ATPases
234	YHR078W	-	2.2941334	3.7838221	4.6151917	Protein of unknown function, has 4 potential transmembrane domains
235	YPL155C	(KIP2)	3.29502679	3.7807978	9.392792	Kinesin-related protein
236	YNR074C	-	4.3061075	3.7638306	5.7991531	Protein with similarity to Bacillus subtilis nitrite reductase (NIRB)
237	YMR303C	(ADH2)	4.56919214	3.7542967	3.1957867	Alcohol dehydrogenase II, glucose-repressed
238	YLR134W	(PDC5)	4.9450653	3.7528169	3.2704832	Pyruvate decarboxylase isozyme 2
239	YKL067W	(YNK1)	4.49102455	3.7325797	3.6497934	Nucleoside diphosphate kinase, responsible for synthesis of all nucleoside triphosphates except ATP
240	YLR136C	(TIS11)	2.88004451	3.7255421	5.1711798	-
241	YDR443C	(SCA1)	2.75733315	3.7068432	6.9331513	-
242	YGL071W	(RCS1)	3.39203358	3.6963077	4.5310166	Regulatory protein involved in IRON uptake
243	YBR293W	-	2.25740646	3.6840827	3.0384171	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
244	YMR324C	-	3.33053542	3.6802526	2.8779503	Protein with similarity to members of the YBL108P/YCR103P/YKL223P family
245	YFL051C	-	2.07690974	3.6611179	4.7476201	Protein with similarity to FLO1P family of proteins
246	YBR276C	(PPS1)	2.35950244	3.6550406	3.4539593	Protein tyrosine phosphatase (PTPase) with dual specificity
247	YFL042C	-	3.57726533	3.6509118	4.5694594	Protein of unknown function, has similarity to YHR080P
248	YPL263C	(KEL3)	4.50871509	3.6484792	3.8498382	Protein with similarity to KEL1P and KEL2P
249	YLR188W	(MDL1)	5.00498919	3.6478982	4.3936321	ATP-binding cassette (ABC) superfamily member, equivalent to a "half-molecule" ABC protein plus an ATP-binding domain



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250	YPR021C	-	2.21061647	3.6466639	3.2312479	Protein with similarity to proteins of the mitochondrial carrier (MCF) family (GB:Z49274)
251	YKL138C	(MRPL31)	3.22538649	3.6454084	4.0488722	Mitochondrial ribosomal protein of the large subunit (YML31)
252	YNL148C	(ALF1)	3.33997835	3.6391378	5.2515594	Alpha-tubulin foldin, cofactor B
253	YLR302C	-	10.5636377	3.6318383	0.3621924	Protein of unknown function
254	YBR298C	(MAL31)	5.44502575	3.6302693	9.8311328	High affinity maltoseH+ symporter (maltose permease) member of the sugar permease family
255	YAR044W	(OSH1)	4.12112011	3.624939	3.8839622	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins
256	YLR120C	(YAP3)	6.14265883	3.6229845	4.4298562	Transcription factor of the basic leucine zipper (BZIP) family, one of eight members of a novel fungal-specific family of BZIP proteins
257	YGR134W	-	2.8756723	3.6189405	1.9505784	Protein of unknown function
258	YMR088C	-	3.01763425	3.574571	2.5742717	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family 2
259	YDR291W	-	4.95353348	3.5637613	2.8803997	Protein with similarity to SGS1P and other DNA helicases
260	YJR017C	(ESS1)	2.98118086	3.5587415	3.2208256	Processing/termination factor, involved in transcription termination or 3'-end processing of pre-mRNA
261	YGL178W	(MPT5)	4.28561965	3.558276	3.3338238	Protein required for high temperature growth, recovery from alpha-factor arrest, and normal lifespan of yeast cells
262	YHR086W	(NAM8)	2.63503306	3.556686	4.1441189	U1 SNRNA-associated protein, essential for meiotic recombination and suppressor of mitochondrial splicing defects, has 3 RNA recognition (RRM) domains
263	YGR178C	(PBP1)	2.95926792	3.5559294	3.6095103	poly(A)-binding protein
264	YBL022C	(PIM1)	4.1993836	3.5255118	3.2518435	Serine protease required for intramitochondrial proteolysis and maintenance of respiratory function, related to E. coli ATP-dependent protease LA
265	YJL083W	-	3.34026267	3.5131828	5.6812601	Protein with similarity to IRS4P
266	YJR053W	-	2.12253894	3.5096202	4.5401439	Protein involved in efficiency of mating
267	YJL175W	-	6.11781731	3.5040684	3.7938536	Protein of unknown function
268	YMR016C	-	3.67893179	3.4720987	3.3111279	-

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269	YLL051C	(FRE6)	2.59520796	3.4643555	4.4566151	Protein with similarity to ferric reductase FRE2P
270	YJL212C	-	4.42206996	3.458335	4.0764022	Protein with similarity to S. pombe ISP4+ which is induced by sexual differentiation
271	YMR019W	(STB4)	3.2576922	3.4414621	3.397646	SIN3P-binding protein, has ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
272	YHL047C	-	3.02606918	3.4089434	3.007693	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
273	YBR038W	(CHS2)	2.03060756	3.3885338	2.8509884	Chitin synthase II, responsible for primary septum disk
274	YLR023C	-	2.68880866	3.3876183	2.5555381	Protein of unknown function
275	YPL009C	-	5.28314415	3.3856037	0.8412905	Protein of unknown function
276	YGL008C	(PMA1)	2.09210526	3.3844005	3.725269	H <sup>+</sup> -transporting P-type ATPase of the plasma membrane, activity is rate-limiting for growth at low pH
277	YMR033W	(ARP9)	3.08586194	3.3800103	2.9005564	Protein with similarity to actin and actin-related proteins ARP1P-ARP10P
278	YLR153C	(ACS2)	3.45528019	3.3785446	3.1285812	Acetyl-CoA synthetase (acetate-CoA ligase)
279	YLL061W	-	10.9366799	3.369477	3.0795633	Protein with similarity to GAP1P and other amino acid permeases
280	YNL192W	(CHS1)	3.72575719	3.358192	3.7457248	Chitin synthase I, has a repair function during cell separation
281	YEL058W	(PCM1)	4.56623631	3.3482618	3.4456437	Hexosephosphate mutase (phosphoacetylglucosamine mutase) (N-acetylglucosamine-phosphate mutase), converts N-acetyl-D-glucosamine 1-phosphate to N-acetyl-D-glucosamine 6-phosphate
282	YLR099C	-	4.72209646	3.3290462	2.9774757	Protein of unknown function
283	YDL057W	-	3.21787484	3.316811	4.4492894	Protein of unknown function
284	YLR195C	(NMT1)	3.4535546	3.3142347	3.1727409	N-myristoyltransferase, adds myristoyl group to N-terminal glycine of certain proteins
285	YAL005C	(SSA1)	3.48582964	3.3068323	2.9388227	Heat shock protein of HSP70 family, cytoplasmic
286	YPL222W	-	2.79485782	3.2974442	2.9997551	Protein of unknown function
287	YJL056C	-	2.36206747	3.2790296	3.3129634	-
288	YKR021W	-	2.33705924	3.269552	2.936447	Protein of unknown function
289	YPL119C	(DBP1)	5.87199247	3.2464223	2.197366	ATP-dependent RNA helicase of dead box family, suppressor of SPP81/DED1

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290	YGL014W	-	3.11296478	3.2294295	3.6382821	Protein with pumilio repeats that is involved with MPT5P in relocalization of SIR3P and SIR4P from telomeres to the nucleolus
291	YER010C	-	11.4713039	3.2179542	2.3807886	Protein of unknown function
292	YJR151C	-	41.4229667	3.2130608	4.5216913	Protein of unknown function
293	YPL207W	-	2.48831068	3.2080219	2.9864022	Protein of unknown function
294	YER130C	-	2.01652303	3.2075344	3.3332725	Protein of unknown function
295	YNR065C	-	2.86361451	3.2060768	7.3945002	Protein with similarity to PEP1P
296	YGL192W	(IME4)	2.89030953	3.170381	6.4784105	Positive transcription factor for IME1 and IME2, mediates control of meiosis by carrying signals regarding mating type (A/alpha) and nutritional status
297	YMR047C	(NUP116)	2.56622055	3.1702234	4.7742052	Nuclear pore protein (nucleoporin) of the GLFG family, may be involved in binding and translocation of nuclear proteins
298	YDR256C	(CTA1)	4.54027942	3.158248	8.0093186	Catalase A (peroxisomal)
299	YDR208W	(MSS4)	2.61164524	3.154316	3.0423151	Potential PI P 5-kinase, multicopy suppressor of STT4 mutation
300	YHR214W	-	4.54013428	3.1513793	5.6325011	Protein of unknown function (YAR066W and YHR214W code for identical proteins)
301	YLR249W	(YEF3)	3.59397167	3.1445334	2.631954	Translation elongation factor EF-3A, member of ATP-binding cassette (ABC) superfamily
302	YNL331C	-	3.44801501	3.1185277	6.5303136	Probable aryl-alcohol reductase
303	YPR115W	-	2.4843458	3.1174643	2.5667848	Protein of unknown function
304	YJL178C	-	2.60257256	3.1121969	2.7705734	Protein of unknown function
305	YAR042W	(SWH1)	18.1940127	3.0992362	6.2488302	Protein of unknown function
306	YDR015C	-	0.09079169	3.0861607	0.3097629	Protein of unknown function
307	YBL067C	(UBP13)	3.41427731	3.0820393	2.4717411	Ubiquitin C-terminal hydrolase
308	YHR072W	(ERG7)	3.5569619	3.0809956	3.4311189	Lanosterol synthase, carries out complex cyclization step of squalene to lanosterol in ergosterol biosynthesis pathway
309	YAL028W	-	9.17485562	3.0726043	3.8109858	Protein of unknown function
310	YIR015W	-	2.80351347	3.066482	3.4328314	Subunit of RNase P
311	YMR308C	(PSE1)	2.69422447	3.0659484	2.6409014	-
312	YOR345C	-	5.73841888	3.0523183	2.2898958	Deoxycytidyl transferase involved in mutagenic translesion DNA synthesis
313	YPL193W	-	3.60415592	3.0500696	2.8450987	Protein of unknown function
314	YFR012W	-	3.31259823	3.0316711	0	Protein of unknown function
315	YPL205C	-	13.258257	3.0208358	0.7999155	Protein of unknown function
316	YDR476C	-	8.1273943	3.0155987	3.8636781	Protein of unknown function
317	YCR052W	(RSC6)	2.2744649	3.0112438	2.6017436	Component of abundant chromatin remodeling complex (RSC)

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318	YGL022W	(STT3)	3.64275733	3.0050118	3.8854905	Oligosaccharyltransferase subunit, member of a complex of eight ER proteins that transfers core oligosaccharide from dolichol carrier to Asn-X-Ser/Thr motif
319	YMR109W	-	19.0544656	3.0044499	5.6886658	-
320	YHR032W	-	9.30722933	2.9855823	4.5560581	Protein of unknown function, member of the major facilitator superfamily(MFS)
321	YLR236C	-	2.6190617	2.9810987	3.7681402	-
322	YOR337W	(TEA1)	2.13152473	2.9790715	4.8228581	TY1 enhancer activator of the GAL4P-type family of DNA-binding proteins
323	YFR055W	-	2.35867872	2.9771983	3.0622139	Protein with similarity to E. coli cystathionine beta-lyase
324	YHR212C	-	4.01639255	2.9769438	4.4451423	Protein identical to YAR060P/RAA19P
325	YLR001C	-	2.77031036	2.9663037	2.7628132	Protein of unknown function
326	YOR034C	-	3.38439363	2.9543526	2.5499862	-
327	YPR076W	-	3.86182393	2.9410933	3.2728075	Protein of unknown function
328	YKL183W	-	2.9718977	2.9334031	5.2561547	Protein of unknown function
329	YBR004C	-	3.05485559	2.9257736	2.8905869	Protein expressed between 3 and 6 hours after transfer to sporulation medium
330	YJR071W	-	3.39019477	2.924417	1.768982	Protein of unknown function
331	YCR084C	(TUP1)	2.40138822	2.9219843	3.2718264	General repressor of transcription (with SSN6P), member of WD (WD-40) repeat family
332	YFR030W	(MET10)	33.6060485	2.9138815	2.0879079	Assimilatory sulfite reductase subunit, flavin-binding (alpha) subunit, part of the sulfate assimilation pathway
333	YKL148C	(SDH1)	2.72554507	2.9036242	2.5317298	Succinate dehydrogenase (ubiquinone) flavoprotein (FP) subunit, converts succinate + ubiquinone to fumarate + ubiquinol in the TCA cycle
334	YER044C	-	3.6669641	2.9002716	2.6807728	Protein of unknown function
335	YLR045C	(STU2)	2.16969039	2.8946579	2.9923107	Component of the spindle pole body
336	YPL226W	-	2.45263084	2.8885678	2.5557944	Protein with similarity to members of the ATP-binding cassette (ABC) superfamily
337	YHR161C	-	2.86345744	2.8873374	2.9469349	-
338	YJR109C	(CPA2)	4.31426739	2.8803515	3.1263529	Carbamoylphosphate synthase (glutamine-hydrolyzing) arginine-specific, large chain
339	YGR250C	-	2.20914388	2.8752914	3.8774955	Protein of unknown function, has three RNA recognition (RRM) domains
340	YLR149C	-	3.39994503	2.8694003	4.6627573	Protein of unknown function

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341	YCL057W	(PRD1)	3.49569406	2.8641379	2.7495149	Proteinase YSCD, saccharolysin, contains zinc metalloendoprotease motif HEXXH
342	YLR114C	-	2.27233205	2.8496505	1.8650501	Protein with weak similarity in the C-terminus to drosophila melanogaster bicaudal-D protein
343	YML075C	(HMG1)	2.71708812	2.8491957	3.2059005	3-hydroxy-3-methylglutaryl-coenzyme A reductase 1, rate limiting enzyme for sterol biosynthesis, converts HMG-COA to mevalonate
344	YLR397C	(AFG2)	2.56801854	2.8469125	2.7385515	Protein of the AAA family of ATPases, has similarity to mammalian valosin-containing protein (VCP)
345	YJR019C	(TES1)	4.07777555	2.8303235	2.0724897	Acyl-CoA thioesterase
346	YBL008W	(HIR1)	7.24580603	2.8284713	2.8866813	Histone transcription inhibitor, required for periodic repression of 3 of the 4 histone gene loci and for autogenous repression of HTA1-HTB1 locus by H2A and H2B
347	YGL062W	(PYC1)	2.649771	2.8279558	3.1059191	Pyruvate carboxylase 1, converts pyruvate to oxaloacetate for gluconeogenesis
348	YPL244C	-	3.43385233	2.8218119	3.3274479	Protein of unknown function
349	YGL001C	-	3.91981575	2.8214816	1.9852785	Protein with similarity to nocardia SP. cholesterol dehydrogenase
350	YMR302C	(PRP12)	2.92335545	2.8146501	2.7190981	-
351	YPL160W	(CDC60)	2.25327101	2.8142723	1.7426948	Leucyl-TRNA synthetase, cytoplasmic
352	YLL024C	(SSA2)	4.09160949	2.8142088	2.4784071	Heat shock protein of HSP70 family, cytoplasmic
353	YEL077C	-	3.20718793	2.8098429	3.9054119	-
354	YMR205C	(PFK2)	2.27470363	2.8050429	2.2843952	Phosphofructokinase beta subunit, part of a complex with PFK1P which carries out key regulatory step in glycolysis
355	YPL114W	-	4.16484234	2.7962162	1.717967	Protein of unknown function
356	YPL221W	-	4.08515832	2.7886642	3.960997	Protein of unknown function
357	YJR137C	(ECM17)	26.5435466	2.787597	2.0763181	Putative sulfite reductase (ferredoxin)
358	YKL164C	(PIR1)	2.11125363	2.7864791	2.3925674	Protein required for tolerance to heat shock, member of the PIR1P/HSP150P/PIR3P family
359	YCL037C	(SRO9)	8.35007693	2.7855748	2.393588	Suppressor of YPT6 null and RHO3 mutations
360	YHR082C	(KSP1)	2.14499054	2.7799591	3.4962633	Serine/threonine kinase that suppresses PRP20 mutant when overproduced
361	YPR074C	-	3.19760669	2.7711859	2.476508	-

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362	YBR184W	(MEL1)	5.06354303	2.7711448	3.5340388	Alpha-galactosidase (melibiase), converts melibiose into galactose + glucose, converts melibiose to galactose and glucose
363	YOL157C	-	2.70064964	2.7668777	3.6204284	Probable alpha-glucosidase
364	YFL066C	-	2.94443276	2.753026	3.5848427	Protein with similarity to other subtelomerically-encoded proteins including YIL177P, YHL050P, and YER190P
365	YLL029W	-	2.22657399	2.7389102	3.1468025	Protein of unknown function
366	YJL198W	-	2.98124683	2.7343513	4.6395823	Protein with strong similarity to PHO87P, member of the phosphate permease family of the major facilitator superfamily (MFS)
367	YDR088C	(SLU7)	2.07293165	2.7339627	2.6876744	Pre-mRNA splicing factor affecting 3' splice site choice, required only for the second catalytic step of splicing
368	YJR132W	(NMD5)	3.2005363	2.7333821	3.208398	Member of the karyopherin-beta family, possibly involved in nuclear transport
369	YIL078W	(THS1)	3.31778832	2.7330794	1.6123939	Threonyl-TRNA synthetase, cytoplasmic, member of Class II family of aminoacyl-TRNA synthetases
370	YGL113W	-	2.33404789	2.7249323	3.3810122	Protein of unknown function
371	YMR086W	-	2.69384376	2.7191747	2.9840404	Protein of unknown function
372	YGL233W	(SEC15)	2.61433498	2.7141295	2.7961427	Component of exocyst complex required for exocytosis
373	YGL144C	-	2.26752066	2.7069494	2.6236889	Protein of unknown function
374	YOR137C	-	3.14249753	2.7031211	4.9526236	Protein of unknown function
375	YJR143C	(PMT4)	2.80130312	2.6954799	2.4879264	Mannosyltransferase (dolichyl phosphate-D-mannose:protein O-D-mannosyltransferase), involved in initiation of O-glycosylation
376	YBR289W	(SNF5)	2.00671327	2.6881295	3.038619	Component of SWI/SNF global transcription activator complex, acts to assist gene-specific activators through chromatin remodeling
377	YNL240C	-	5.13894557	2.685901	3.523963	Protein with similarity to kluyveromyces MARX. LET1 protein
378	YML013W	-	3.62672833	2.6831604	2.9292996	Protein of unknown function
379	YKL168C	-	2.43589311	2.6791837	3.1896257	-
380	YGL151W	(NUT1)	2.47823061	2.6787971	2.5683618	Protein that affects expression of HO
381	YNL197C	(WHI3)	2.51493336	2.6764555	3.4233233	Protein involved in regulation of cell size, has 1 RNA recognition (RRM) domain
382	YMR192W	-	2.18376269	2.6732126	2.7489187	Protein with similarity to mouse TBC1 protein

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383	YAL038W	(CDC19)	2.63679951	2.6714535	2.7525692	-
384	YEL075C	-	5.12225893	2.6632638	3.778537	Protein with similarity to other subtelomerically-encoded proteins including YHL049P, YIL177P, and YJL225P
385	YHR219W	-	3.76398139	2.6619567	4.0207342	Protein with similarity to other subtelomerically-encoded proteins
386	YJL069C	-	2.65731007	2.6517254	2.6568606	Protein of unknown function
387	YLR125W	-	6.28348756	2.642933	3.1335402	Protein of unknown function
388	YML035C	(AMD1)	2.23864371	2.6401405	1.6690608	AMP deaminase, converts AMP to IMP and ammonia
389	YMR165C	(SMP2)	2.58642399	2.6310411	3.3572604	Protein whose deletion causes increased plasmid stability
390	YDL223C	-	3.16684859	2.6240147	2.2340877	Protein of unknown function
391	YLR138W	-	2.69090586	2.6158483	2.9821754	-
392	YAR020C	-	3.79173888	2.6111125	2.0234222	-
393	YLR337C	(VRP1)	6.57336326	2.6027011	3.7504037	Proline-rich protein verprolin, involved in cytoskeletal organization and cellular growth
394	YLR060W	(FRS1)	2.61550639	2.5992071	1.9335426	Phenylalanyl-TRNA synthetase, alpha subunit, cytoplasmic
395	YLL013C	-	2.93447915	2.5901954	4.1297767	Protein with similarity to drosophila pumilio protein
396	YIR003W	-	2.41363594	2.5863745	2.874494	Protein with similarity to E. coli and Bacillus subtilis mind, has potential coiled-coil region
397	YIL137C	-	2.27968603	2.5792356	1.9624104	Protein with similarity to aminopeptidases
398	YBL081W	-	2.34404421	2.573205	3.2079939	Protein with 37% identity to drosophila L not protein
399	YOR171C	-	3.59659097	2.5718305	2.4035974	-
400	YPL237W	(SUI3)	2.5966981	2.5628077	2.5479456	Translation initiation factor EIF2beta subunit
401	YHR142W	-	3.52383057	2.5597096	2.9887896	Protein of unknown function
402	YLL012W	-	3.25020683	2.550591	2.7451737	Protein with similarity to human triacylglycerol lipase
403	YFR025C	(HIS2)	2.5112362	2.5457991	2.8789156	Histidinol phosphatase
404	YGR240C	(PFK1)	2.24103063	2.5388997	2.4938739	Phosphofructokinase alpha subunit, part of a complex with PFK2P which carries out A key regulatory step in glycolysis
405	YPL101W	-	4.18961695	2.5351198	2.6201803	Protein of unknown function
406	YOR127W	(RGA1)	3.85804733	2.5316649	2.5697341	RHO-type GTPase-activating protein (GAP) for CDC42P
407	YBR088C	(POL30)	2.5383718	2.5276319	4.0628861	Proliferating cell nuclear antigen (PCNA), required for DNA synthesis and DNA repair
408	YBR295W	(PCA1)	4.16669535	2.525791	1.1221384	P-type copper-transporting ATPase
409	YCL044C	-	2.35958836	2.519608	3.263571	Protein of unknown function

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410	YBR110W	(ALG1)	2.23384099	2.5141215	3.2250999	Beta-mannosyltransferase involved in N-glycosylation (transfers MAN from GDP-MAN to DOL-PP-GLCNAC2)
411	YGR175C	(ERG1)	6.02726287	2.5103577	1.8132661	Squalene monooxygenase (squalene epoxidase), enzyme of the ergosterol biosynthesis pathway
412	YLR116W	-	2.98116702	2.5079761	3.9707409	-
413	YCR068W	-	3.32107678	2.4920381	3.6811994	Protein of unknown function
414	YJR105W	-	2.20476096	2.4908887	1.7029385	Protein with similarity to ribokinase
415	YKL157W	(APE2)	2.18209838	2.4866194	2.093134	Aminopeptidase II (YSCII), plays a nutritional role in releasing leucine from peptides externally cleaved at leucine
416	YFR009W	(GCN20)	2.63782118	2.4859544	2.1613378	Component of a protein complex required for activation of GCN2P protein kinase in response to amino acid starvation, member of ATP-binding cassette (ABC) superfamily
417	YDR211W	(GCD6)	2.22567451	2.4835485	1.8240639	Translation initiation factor EIF2B (guanine nucleotide exchange factor), 81 KDA (beta) subunit
418	YAR060C	-	4.88485967	2.482682	4.6114571	Protein identical to YHR212P, has a predicted mitochondrial transit peptide
419	YJL187C	(SWE1)	2.01161328	2.4809757	2.6294797	Serine/tyrosine dual-specificity protein kinase able to phosphorylate CDC28P on tyrosine and inhibit its activity
420	YDR387C	-	2.3225348	2.4746572	3.0481024	Protein with similarity to ITR1P and ITR2P
421	YDR251W	(PAM1)	2.09471237	2.4744652	2.3344613	Coiled-coil protein and multicopy suppressor of loss of PP2A (genes PPH21, PPH22, and PPH3)
422	YJL172W	(CPS1)	2.4464951	2.473092	2.228723	GLY-X carboxypeptidase YSCS, involved in nitrogen metabolism
423	YMR277W	(FCP1)	2.51675116	2.466666	2.2346158	TFIIF-interacting component of the C-terminal domain phosphatase
424	YDL047W	(SIT4)	2.40214863	2.4572974	2.7529791	Protein serine/threonine phosphatase involved in cell cycle regulation, member of the PPP family of protein phosphatases and related to PP2A phosphatases
425	YML117W	-	2.2473701	2.4482108	2.8054783	Protein of unknown function, contains an ATP/GTP-binding site motif A (P-loop)
426	YHR039C-A	-	2.49103418	2.4469729	1.7368373	-
427	YLL003W	(SFI1)	3.03031186	2.4467012	2.2685901	Protein of unknown function



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428	YKR048C	(NAP1)	3.02222721	2.4404483	3.002619	Nucleosome assembly protein that plays a role in assembly of histones into octamer, required for full expression of CLB2P functions
429	YOR197W	-	2.87645711	2.438206	1.9784081	Protein of unknown function
430	YEL046C	(GLY1)	2.40664526	2.4369367	2.6795853	Protein required for glycine prototrophy in SHMT1 SHMT2 double mutant
431	YJL029C	-	2.36823878	2.43429	2.3384644	Protein of unknown function, has similarity to C. elegans
432	YOR233W	(KIN4)	3.52231883	2.4312627	3.0678435	hypothetical protein T05G5.8 Serine/threonine protein kinase related to KIN1P and KIN2P, catalytic domain is most related to SNF1P
433	YOR299W	(BUD7)	2.16058794	2.4312223	2.9585581	Protein required for bipolar budding pattern
434	YHR218W	-	2.37694362	2.4297245	4.1990669	Protein with similarity to other subtelomerically-encoded proteins including YHR219P and YFL065P, probable pseudogene
435	YGL026C	(TRP5)	3.92053304	2.4267316	2.4752996	Tryptophan synthase, last (fifth) step in tryptophan biosynthesis pathway
436	YJL017W	-	2.745014	2.4179146	2.8613495	Protein of unknown function
437	YNL161W	-	4.7525671	2.4161417	2.324762	Serine/threonine protein kinase of unknown function
438	YOR141C	(ARP8)	5.68817037	2.4122798	1.7395537	Protein with similarity to actin and actin-related proteins ARP1P-ARP10P
439	YAL042W	-	2.84377325	2.4057529	3.7961408	Protein of unknown function, has 2 potential transmembrane domains
440	YGR270W	(YTA7)	2.68803581	2.4056715	1.945755	Protein with similarity to members of the AAA family of ATPases
441	YBR119W	(MUD1)	2.83912216	2.4051525	1.3987642	U1 SNRNP A protein (SNRNA-associated protein) with 2 RNA recognition (RRM) domains
442	YDR052C	(DBF4)	6.85835185	2.4036928	1.5834976	Regulatory subunit for CDC7P protein kinase, required for G1/S transition
443	YEL069C	(HXT13)	2.69020108	2.4013304	3.6711431	Protein with strong similarity to hexose transporters, member of the sugar permease family
444	YDR285W	(ZIP1)	8.03633767	2.3921886	0.2216256	Structural protein of the synaptonemal element central element, has predicted coiled-coil domain
445	YJL047C	-	2.8960182	2.3885065	2.0814157	Protein with similarity to clathrin heavy chain in one domain
446	YKL101W	(HSL1)	4.2235071	2.3780286	2.4485279	Serine/threonine protein kinase that interacts genetically with histone mutations

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
447	YIL143C	(SSL2)	2.16202858	2.3668818	1.9944618	DNA helicase component of RNA polymerase transcription initiation factor TFIIF (factor B)
448	YBR182C	-	3.15043584	2.3653183	2.517131	-
449	YER189W	-	2.65287612	2.3630614	5.1724275	Protein with similarity to subtelomerically-encoded proteins including YIL177P, YHL049P, and YJL225P
450	YLR194C	-	3.11287981	2.3617044	2.923307	Protein of unknown function
451	YGR160W	-	2.13853989	2.3577684	1.8132562	Protein of unknown function
452	YGR258C	(RAD2)	2.06944636	2.3572245	2.1751698	Structure-specific single-stranded DNA endonuclease of the nucleotide excision repairosome
453	YGR162W	(TIF4631)	2.28099935	2.3554791	1.7039222	MRNA CAP-binding protein (EIF4F) 150K subunit
454	YJR036C	-	3.21027204	2.3546452	5.0712893	Possible ubiquitin-protein ligase (E3)
455	YGR124W	(ASN2)	3.37829988	2.3505148	2.4742017	Asparagine synthetase (L-aspartate: L-glutamine amidoligase [AMP-forming]), ASN1P and ASN2P are isozymes
456	YDL180W	-	2.20643197	2.3467843	1.8047293	Protein of unknown function
457	YDR266C	-	3.29383065	2.3411759	2.3118864	Protein of unknown function
458	YAR073W	-	7.67257484	2.3325262	1.6890618	Protein with strong similarity to PUR5P, may be an inosine-5'-monophosphate dehydrogenase
459	YPL048W	(CAM1)	2.18528771	2.3294863	3.3106924	-
460	YEL030W	(ECM10)	1.99868799	2.3236082	2.3835153	Protein possibly involved in cell wall structure or biosynthesis
461	YLL058W	-	6.13836096	2.3223158	2.3541199	Protein with similarity to neurospora crassa O-succinylhomoserine (thiol)-lyase
462	YJR010W	(MET3)	8.36384636	2.3172147	1.5113084	ATP-sulfurylase (sulfate adenylyltransferase)
463	YER110C	(KAP123)	3.02732098	2.3160572	1.8042941	Karyopherin-beta, involved in nuclear import of ribosomal proteins
464	YGL063W	(PUS2)	2.17517427	2.3124794	4.1754638	Pseudouridine synthase
465	YPL184C	-	3.3404012	2.3122475	2.2563666	Protein of unknown function
466	YGR254W	(ENO1)	2.05650599	2.3095639	1.9054756	Enolase 1 (2-phosphoglycerate dehydratase), converts 2-phospho-D-glycerate to phosphoenolpyruvate in glycolysis
467	YIL108W	-	3.15926869	2.3086561	2.5782072	Protein of unknown function
468	YDR388W	(RVS167)	2.34115713	2.3058518	2.6912527	Protein with A SH3 domain that affects actin distribution and bipolar budding
469	YNL323W	-	2.29668952	2.3038327	2.0645985	Protein with similarity to YCX1P
470	YBL076C	(ILS1)	2.31635893	2.3036041	1.7634202	Isoleucyl-TRNA synthetase
471	YLR217W	-	2.57939547	2.2859565	1.6611523	Protein of unknown function
472	YGR294W	-	8.48668724	2.2857763	1.7132102	Protein of the PAU1 family
473	YDL070W	-	2.16064033	2.2854538	3.7599153	-

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
474	YOL044W	-	2.15373467	2.2849315	2.1736446	-
475	YGL145W	(TIP20)	4.18903489	2.2829973	1.6161221	Cytoplasmic protein that interacts physically with SEC20P, required for ER to Golgi transport
476	YLR044C	(PDC1)	2.21772333	2.2774972	1.9431592	Pyruvate decarboxylase isozyme 1
477	YNR013C	-	2.0080141	2.2770842	2.4893728	Protein with similarity to PHO87P and YJL198P, member of the phosphate permease family of the major facilitator superfamily (MFS)
478	YML049C	-	2.08547393	2.2761395	2.0329879	-
479	YDR221W	-	2.53153283	2.2731861	1.8131644	Protein with similarity to the beta subunit of human glucosidase II
480	YMR135C	-	4.75727106	2.2636411	4.3609747	Protein of unknown function
481	YKR001C	(VPS1)	2.48277065	2.2630712	1.5678763	Vacuolar sorting protein, member of the dynamin family of GTPases
482	YLR413W	-	2.80009402	2.2629262	2.3695083	Protein of unknown function
483	YDR122W	(KIN1)	2.0434064	2.2623436	2.3432635	Serine/threonine protein kinase, related to KIN2P and S. pombe KIN1
484	YIL154C	(IMP2')	2.216207	2.2548739	2.2466776	-
485	YKL068W	(NUP100)	2.2598003	2.2529093	2.7012733	Nuclear pore protein (nucleoporin) of the GLFG family, may be involved in binding and translation of proteins during nucleocytoplasmic transport
486	YHR190W	(ERG9)	2.81318531	2.2475123	1.7238705	Squalene synthetase (farnesyl-diphosphate farnesyltransferase), acts at a branch point in the isoprenoid biosynthesis pathway
487	YGL179C	-	4.83814707	2.2398396	3.8786749	Serine/threonine protein kinase with similarity to ELM1P and KIN82P
488	YOL017W	-	3.01741322	2.2303862	2.2459064	Protein of unknown function
489	YHR189W	-	2.0021212	2.22911	2.2289936	Putative peptidyl-TRNA hydrolase (PTH)
490	YNL208W	-	3.64860898	2.2181817	2.5247363	Protein of unknown function
491	YHR041C	(SRB2)	2.27216109	2.2178582	2.4847273	Component of the RNA polymerase II holoenzyme and Kornberg's mediator (SRB) subcomplex
492	YPR080W	(TEF1)	2.50402057	2.2115095	1.8587879	Translation elongation factor EF-1alpha (TEF1 and TEF2 code for identical proteins)
493	YBR229C	(ROT2)	2.45186053	2.2034499	2.1844611	Catalytic (alpha) subunit of glucosidase II
494	YGR262C	-	2.83275613	2.2029336	1.9251756	Protein with similarity to apple tree calcium/calmodulin-binding protein kinase PIR:JQ2251

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
495	YER144C	(UBP5)	3.38126089	2.1994294	2.7106303	Ubiquitin-specific protease (ubiquitin C-terminal hydrolase), homologous to DOA4P and human TRE-2
496	YDR264C	(AKR1)	3.13151279	2.1983967	2.7516536	Ankyrin repeat-containing protein that has an inhibitory effect on signaling in the pheromone pathway
497	YLR427W	-	2.24985411	2.1938243	2.5059695	Protein of unknown function
498	YLR374C	-	2.26923061	2.1927227	2.6395044	Protein of unknown function
499	YMR092C	(AIP1)	2.2241966	2.1917074	2.1939749	Actin interacting protein, has 4 WD (WD-40) repeats
500	YDR294C	-	2.20085342	2.1899557	2.3333139	-
501	YMR296C	(LCB1)	2.1334221	2.1891645	1.9030014	Component of serine C-palmitoyltransferase, first step in biosynthesis of long-chain base component of sphingolipids
502	YKR039W	(GAP1)	1.99105648	2.1881751	1.2556866	General amino acid permease, proton symport transporter for all naturally-occurring L-amino acids, 4-aminobutyric acid (GABA), ornithine, citrulline, some D-amino acids, and some toxic analogs
503	YDR422C	(SIP1)	2.62373247	2.1870761	2.0836347	Multicopy suppressor of SNF1, related to GAL83P/SPM1P and SPM2P
504	YMR080C	(NAM7)	2.82340116	2.1828046	2.1714828	Protein involved with NMD2P and UPF3P in decay of MRNA containing nonsense codons
505	YBL106C	-	2.38138747	2.1809814	2.7798326	-
506	YEL043W	-	3.44125375	2.1784956	2.8076042	Protein of unknown function
507	YBR222C	(FAT2)	5.13679804	2.1781103	3.0936394	Peroxisomal AMP-binding protein
508	YDR004W	(RAD57)	2.26389978	2.1754266	2.076582	Component of recombinosome complex involved in meiotic recombination and recombinational repair, with RAD55P promotes DNA strand exchange by RAD51P recombinase
509	YHR174W	(ENO2)	2.38714668	2.1702816	1.9697417	Enolase 2 (2-phosphoglycerate dehydratase), converts 2-phospho-D-glycerate to phosphoenolpyruvate in glycolysis
510	YER043C	(SAH1)	3.73200717	2.1669937	1.6246235	Adenosylhomocysteinase (S-adenosylhomocysteine hydrolase)
511	YKR012C	-	2.41358469	2.1555775	1.1414615	Protein of unknown function
512	YOL007C	-	3.17872347	2.1529948	1.2712945	-
513	YMR220W	(ERG8)	2.68816133	2.1489328	2.0693924	Phosphomevalonate kinase, converts mevalonate-5-phosphate to mevalonate pyrophosphate, involved in isoprene and ergosterol biosynthesis pathways

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514	YDR062W	(LCB2)	2.54448949	2.1430094	1.9627647	Subunit of serine C-palmitoyltransferase, first step in sphingolipic biosynthesis, and suppressor of calcium-sensitivity of CSG2
515	YAL048C	-	5.02313141	2.1384748	4.221132	Protein with weak similarity to RAS1P, RAS2P, and other GTP-binding proteins of the RAS superfamily
516	YBL111C	-	2.17340644	2.1313903	3.8030907	-
517	YJL108C	-	4.56646166	2.1302533	2.8609713	Protein of unknown function, contains 8 potential transmembrane domains
518	YJL141C	(YAK1)	2.80000608	2.1277388	2.8291776	Serine/threonine protein kinase, negative regulator of cell growth acting in opposition to CAMP-dependent protein kinase A
519	YJL102W	(MEF2)	2.08592026	2.1220696	1.6098307	Mitochondrial translation elongation factor, promotes GTP-dependent translocation of nascent chain from A-site to P-site of ribosome
520	YDL174C	(DLD1)	2.28050309	2.1220649	2.4305801	D-lactate dehydrogenase (cytochrome), (D-lactate ferricytochrome C oxidoreductase) (D-LCR), mitochondrial
521	YMR011W	(HXT2)	7.25080973	2.1188378	1.6420019	High-affinity hexose transporter, member of sugar permease family
522	YLR129W	(DIP2)	3.36115373	2.1126408	2.0325416	DOM34P-interacting protein, has WD (WD-40) repeats
523	YML008C	(ERG6)	2.51872662	2.1091692	1.7889829	S-adenosylmethionine delta-24-sterol-C-methyltransferase, carries out methylation of zymosterol as part of the ergosterol biosynthesis pathway
524	YGL245W	-	2.30162026	2.1065078	1.4267053	Glutamyl-TRNA synthetase, member of the Class I aminoacyl TRNA synthetase family
525	YGL024W	-	2.67631735	2.1046757	1.4610387	Protein of unknown function
526	YHL027W	(RIM101)	2.57210755	2.1033157	2.5927892	Zinc-finger protein involved in induction of IME1
527	YGR281W	(YOR1)	4.18259907	2.0935061	2.3634092	Oligomycin-resistance factor, member of the ATP-binding cassette (ABC) superfamily
528	YIL175W	-	2.10803474	2.0859355	2.4771166	-
529	YHL019C	(APM2)	1.9986708	2.0848729	3.0618718	Clathrin-associated protein (AP) complex, medium subunit
530	YAL019W	(FUN30)	5.19927199	2.0806959	1.7340212	-
531	YGL112C	(TAF60)	2.21463331	2.0765265	2.1891308	Component of TAF(II) complex (TBP-associated protein complex) required for activated transcription by RNA polymerase II

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
532	YNL218W	-	2.28887465	2.0761	1.6749939	Protein with similarity to E. coli DNA polymerase III gamma and TAU subunits
533	YML058C-A	-		217.969407	2.0723568	3.2869214
534	YOL156W	(HXT11)	5.12784966	2.0709411	2.2192118	Low-affinity glucose permease
535	YGR218W	(CRM1)	2.32581989	2.0675233	1.5505702	Exportin, beta-karyopherin
536	YGR296W	-	3.15948331	2.0664535	3.7402619	Protein with similarity to other subtelomerically-encoded proteins including YER190P (YPL283 and YGR296W code for identical proteins)
537	YLR176C	-	2.54329087	2.0627475	1.4892288	-
538	YDL229W	(SSB1)	5.21935107	2.0615889	2.0067653	Heat shock protein of HSP70 family involved in the translational apparatus
539	YER034W	-	2.57654853	2.0562947	1.9025056	Protein of unknown function
540	YKR050W	(TRK2)	2.23638067	2.056259	4.703529	Potassium transporter of the plasma membrane, moderate affinity, member of the potassium permease family of the major facilitator superfamily
541	YIL113W	-	7.07756282	2.0539759	2.28618	Dual-specificity protein phosphatase
542	YCR023C	-	2.01851078	2.0520751	2.2109695	Member of major facilitator superfamily (MFS) multidrug-resistance protein family 2
543	YMR069W	-	4.45745957	2.0520592	0	Protein of unknown function
544	YAL020C	(ATS1)	3.02597511	2.050802	2.0781706	Protein with similarity to human RCC1 protein, suppressor of mutations in alpha tubulin
545	YNL256W	-	3.16308725	2.045577	1.8697374	Protein with similarity to bacterial dihydropteroate synthase
546	YMR124W	-	2.65610298	2.0431312	2.2988806	Protein of unknown function, has potential coiled-coil region (GB:Z49273)
547	YOR162C	-	2.4478098	2.0361958	2.1075035	-
548	YOR353C	-	2.20965265	2.0220258	1.7471747	Protein with weak similarity to adenylate cyclases
549	YPL028W	(ERG10)	2.86559138	2.0185951	1.6989337	Acetyl-CoA acetyltransferase (acetoacetyl-CoA thiolase), first step in mevalonate/sterol pathway
550	YIL114C	(POR2)	2.24322702	2.0152799	2.367678	Outer mitochondrial membrane porin (voltage-dependent anion-selective channel)
551	YDL029W	(ACT2)	2.07186888	2.0140172	1.810394	-
552	YDL143W	(CCT4)	2.3041307	2.0128325	1.6478427	Component of chaperonin-containing T-complex (TCP ring complex, TRIC), homologous to mouse CCT4
553	YPL267W	-	2.06501413	2.0119076	1.6761922	Protein of unknown function
554	YOL105C	-	2.79225712	2.0026061	2.23737	-

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
555	YML004C	(GLO1)	2.19630894	2.0015677	1.7985136	Glyoxalase I, converts methylglyoxal and glutathione into S-D-lactoylglutathione
556	YMR266W	-	2.47393267	1.991188	1.727182	Protein of unknown function, probable integral membrane glycoprotein
557	YPL194W	-	2.87006368	0.4961465	1.5346869	-
558	YOR152C	-	2.74047761	0.4915256	0.2221023	Protein of unknown function
559	YDR242W	(AMD2)	8.28951711	0.4819032	0.9215489	Protein with similarity to amidases
560	YFL054C	-	7.43223753	0.4793582	0.6136582	Protein with similarity to FPS1P and YPR192P, member of MIP family of transmembrane channels
561	YAR068W	-	3.24259317	0.479021	1.2297001	Protein with similarity to ICWP protein
562	YAL001C	(TFC3)	2.94740587	0.4742746	1.1915566	RNA polymerase transcription initiation factor TFIIC (TAU), 138 KDA subunit
563	YLR454W	-	5.72921213	0.4716283	1.641906	Protein of unknown function
564	YDL020C	(SON1)	2.27378766	0.4591519	0.8208918	-
565	YMR225C	(MRPL44)	0.19372389	0.4430311	0.4019617	Mitochondrial ribosomal protein of the large subunit (YMR44)
566	YJR038C	-	9.06373624	0.4422872	4.1801655	Protein of unknown function
567	YDR380W	-	0.1136124	0.4417559	0.8241167	Protein with similarity to pyruvate decarboxylase, pyruvate oxidase, acetolactate synthase (large subunit), and other enzymes that require thiamine pyrophosphate
568	YKL170W	(MRPL38)	0.20347891	0.4296401	0.4533368	Mitochondrial ribosomal protein of the large subunit (YML38) (E. coli L14), belongs to the L14 family of prokaryotic ribosomal proteins
569	YGR248W	(SOL4)	0.17664863	0.4293198	0.4062793	Protein of unknown function
570	YER058W	(PET117)	0.18996331	0.4289442	0.4202828	Protein involved in assembly of cytochrome oxidase
571	YBR039W	(ATP3)	0.18787084	0.4197886	0.2837245	F1-gamma ATP synthase
572	YDL102W	(CDC2)	17.5853214	0.4169873	0.0258767	-
573	YJR153W	-	3.65551445	0.4116558	0.6086987	-
574	YMR188C	-	0.20743995	0.4113817	0.3381207	Protein with similarity to 30S ribosomal proteins (S17)
575	YBR244W	-	0.16093632	0.4035137	0.3438917	Protein with similarity to glutathione peroxidase
576	YDR523C	(SPS1)	10.8815611	0.4014712	0.3371725	Serine/threonine protein kinase involved in middle/late stage of meiosis
577	YDL031W	-	2.0561223	0.3989968	0.6791327	Protein with similarity to RNA helicases of dead/DEAH box family
578	YER109C	(FLO8B)	2.33584341	0.3826502	2.0529509	-

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
579	YIR017C	(MET28)	2.97658904	0.3775372	0.3008895	Transcriptional activator of the basic leucine zipper (BZIP) family, works with MET4P and CBF1P to regulation sulfur amino acid metabolism
580	YDL016C	-	3.9417341	0.374232	0.2672688	Protein of unknown function
581	YIR028W	(DAL4)	2.5006493	0.3741716	3.0010653	Allantoin permease, member of the uracil/allantoin permease family of the major facilitator superfamily (MFS)
582	YOR124C	(UBP2)	2.8382974	0.3622925	0.3859773	Ubiquitin-specific protease (ubiquitin C-terminal hydrolase), cleaves at the C-terminus of ubiquitin
583	YBL108W	-	0.1900473	0.3575329	0.5467376	Protein of unknown function
584	YDR259C	-	5.62713626	0.3429355	0.2335082	-
585	YDR253C	(MET32)	2.86314943	0.3397175	0.3279043	Zinc-finger protein involved in transcriptional regulation of methionine metabolism
586	YJL196C	(ELO1)	0.17135352	0.3378086	0.3752547	Fatty acid elongation protein involved in elongation of tetradecanoic acid to hexadecanoic acid
587	YDR141C	-	0.09160554	0.3290633	0.1693929	Protein of unknown function, member of the major facilitator superfamily (MFS)
588	YBR069C	(VAP1)	3.0181038	0.3157547	1.2268269	Amino acid permease for valine, leucine, isoleucine, tyrosine, and tryptophan
589	YOR314W	-	2.65430513	0.2917342	0.3312621	Protein of unknown function
590	YDL068W	-	0.11556176	0.2684108	0.1521109	Protein of unknown function
591	YPL136W	-	2.17418921	0.2530647	3.1708409	Protein of unknown function
592	YGL034C	-	0.1411795	0.2524039	0.3723439	Protein of unknown function
593	YLR162W	-	4.13626663	0.2515583	0.6851592	Protein of unknown function
594	YMR193C-A	-	3.34099753	0.2354896	0.3596816	-
595	YMR146C	(TIF34)	5.0351989	0.2248204	0.7193538	Translation initiation factor EIF3, P39 subunit, has 2 WD (WD-40) repeats
596	YFL012W	-	71.9436495	0.2235373	1.5215902	Protein of unknown function
597	YER096W	-	7.21258235	0.1766673	0.4170679	-
598	YNR071C	-	2.01488788	0.1446196	0.0535063	Protein with similarity to UDPglucose 4-epimerase
599	YLR419W	-	0.20769335	0.1102431	0.9141258	Protein with similarity to several pre-mRNA splicing factors
600	YKL105C	-	3.23146223	0.086572	5.0836556	Protein of unknown function
601	YLR142W	(PUT1)	2.2907881	0.0854218	0.6671487	Proline oxidase, first step in synthesis of glutamate from proline
602	YDL239C	-	7.81000565	0.0417738	0.347901	Protein of unknown function
603	YHR137W	(ARO9)	0.07724918	0.0347684	0.0703134	Aromatic amino acid aminotransferase II
604	YDR374C	-	17.25276	0	4.6059679	Protein of unknown function



Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
605	YIL100W	-	9.97598883	0	2.8122773	Protein of unknown function, questionable ORF
606	YPL025C	-	9.52247441	0	20.22382	Protein of unknown function
607	YOR072W	-	7.48662389	0	6.2287404	Protein of unknown function
608	YNL242W	-	6.47720448	0	2.5753253	Protein of unknown function
609	YIR027C	(DAL1)	5.64113227	0	0	Allantoinase, first step in the degradation of allantoin as a secondary nitrogen source
610	YOR139C	-	5.46648132	0	11.760995	Transcription factor with domains homologous to MYC oncoprotein and yeast HSF1P, required for normal cell surface assembly and flocculence
611	YEL019C	(MMS21)	3.34008483	0	2.069236	Protein involved in DNA repair
612	YDL132W	(cdc53)	3.16426832	0	0.1467847	-
613	YOR177C	-	2.97842594	0	0.435871	Protein of unknown function
614	YML042W	(CAT2)	2.76437696	0	16.65885	Carnitine O-acetyltransferase, peroxisomal and mitochondrial
615	YER044C-A	(MEI4)	2.5971776	0	0	Protein required early in meiosis for meiotic recombination, chromosome synapsis, and viable spore formation
616	YGR083C	(GCD2)	2.32134339	0	0	Translation initiation factor EIF2B (guanine nucleotide exchange factor), 71 KDA (delta) subunit
617	YAR030C	-	2.06301879	0	0	Protein of unknown function, probable non-coding ORF
618	YJR157W	-	0.2073771	0	0.6711879	Protein of unknown function
619	YHR217C	-	0.2061042	0	0.549346	Protein of unknown function
620	YKL100C	-	0.12715731	0	40.399169	Protein of unknown function

\* Table Headings:

Clone ID: A clone ID designation number.

Alias: Alternative gene names used in the literature. This information is provided by YPD™, Hodges *et al. Nucl. Acids Res.* 27: 69-73 (1999), the entirety of which is herein incorporated by reference.

CJ-4hr/LP-4hr: Expression level in the mutant CJ517 as compared with the respective wild type strain LPY9 at 4hr sampling of log phase growth of yeast (ratio of mutant expression level/control expression level). CJ refers to the mutant CJ517 (The mutant is defective in the gene (ERG11) codes for C14 demethylase enzyme in the sterol biosynthetic pathway). LP refers to the

respective wild type strain LPY9, used to compare the gene expression profile with the mutant.

K-50/CK: Expression level in the wild type yeast LPY9, at 2 hr after treatment with 50micro gram/ml ketoconazole as compared to the wild type LPY9 strain without ketoconazole treatment (ratio of treatment expression level/control expression level). K refers to ketoconazole treatment. The clones listed in Table 2 are either up or down regulated in the mutant (CJ517) as well as in response to ketoconazole treatment.

K-100/CK: Expression level in the wild type yeast LPY9, at 2 hr after treatment with 100micro gram/ml ketoconazole as compared to the wild type LPY9 strain without ketoconazole treatment (ratio of treatment expression level/control expression level).

Gene Description: Description of the clone listed in column 1.

Table 3, below, lists the RNAs from Table 2 that correspond to genes or structural regions implicated in transcription regulation.

**Table 3\***

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
30	YOR237W	(HES1)	134.648161	1417.62621	1358.12348	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins
42	YDR213W	-	18.2079478	32.1360646	58.3586116	Protein with similarity to transcription factors, has ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
74	YGR177C	(ATF2)	3.7081426	11.830167	12.5552685	Alcohol O-acetyltransferase
75	YFR034C	(PHO4)	14.8112083	11.2160731	20.8445145	Basic helix-loop-helix (BHLH) transcription factor required for expression of phosphate pathway, hyperphosphorylation by PHO80P-PHO85P cyclin-dependent protein kinase complex causes inactivation

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
83	YOL067C	(RTG1)	30.4142081	10.0270648	27.3663295	Basic helix-loop-helix (BHLH) transcription factor involved in inter-organelle communication between mitochondria, peroxisomes, and nucleus
100	YJL127C	(SPT10)	4.01528284	7.83944269	10.0960266	Protein that amplifies the magnitude of transcriptional regulation at various loci
111	YMR037C	(MSN2)	6.80686734	6.42359685	7.66129891	Zinc-finger transcriptional activator for genes involved in the multistress response and genes regulated through SNF1P
118	YCR048W	(ARE1)	9.11370518	6.1039374	10.5312906	Acyl-CoA:sterol acyltransferase (sterol-ester synthetase)
131	YAL013W	(DEP1)	8.79366086	5.54633863	6.42500999	Regulator of phospholipid metabolism
132	YIL084C	(SDS3)	1.99582364	5.54306878	6.90742248	Suppressor of silencing defect
157	YKR034W	(DAL80)	3.91750209	5.0436172	7.28385659	GATA-type zinc finger transcriptional repressor for allantoin and 4-aminobutyric acid (GABA) catabolic genes
172	YLR098C	(CHA4)	2.05280928	4.75643469	5.58664651	Zinc-finger protein required for activation of CHA1, has A ZN[2]-CYS[6] fungal-type binuclear cluster domain
180	YDR389W	(SAC7)	3.89197011	4.56095992	4.31431086	GTPase-activating protein for RHO1P
202	YDL088C	(ASM4)	4.39685251	4.17572645	3.32103404	Suppressor of temperature-sensitive mutations in POL3P (DNA polymerase delta)
206	YBL005W	(PDR3)	3.75060207	4.14490535	6.08273054	Transcription factor related to PDR1P, contains A ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
242	YGL071W	(RCS1)	3.39203358	3.69630773	4.53101664	Regulatory protein involved in iron uptake
255	YAR044W	(OSH1)	4.12112011	3.624939	3.88396219	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins
256	YLR120C	(YAP3)	6.14265883	3.62298451	4.42985615	Transcription factor of the basic leucine zipper (BZIP) family, one of eight members of a novel fungal-specific family of BZIP proteins
260	YJR017C	(ESS1)	2.98118086	3.55874146	3.22082555	Processing/termination factor, involved in transcription termination or 3'-end processing of pre-mRNA

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
271	YMR019W	(STB4)	3.2576922	3.44146214	3.39764598	SIN3P-binding protein, has ZN[2]-CYS[6] fungal-type binuclear cluster domain in the N-terminal region
278	YLR153C	(ACS2)	3.45528019	3.37854457	3.12858117	Acetyl-CoA synthetase (acetate-CoA ligase)
289	YPL119C	(DBP1)	5.87199247	3.24642228	2.19736599	ATP-dependent RNA helicase of dead box family, suppressor of SPP81/DED1
290	YGL014W	-	3.11296478	3.22942947	3.6382821	Protein with pumilio repeats that is involved with MPT5P in relocalization of SIR3P and SIR4P from telomeres to the nucleolus
296	YGL192W	(IME4)	2.89030953	3.17038103	6.47841053	Positive transcription factor for IME1 and IME2, mediates control of meiosis by carrying signals regarding mating type (A/alpha) and nutritional status
297	YMR047C	(NUP116)	2.56622055	3.17022339	4.77420515	Nuclear pore protein (nucleoporin) of the GLFG family, may be involved in binding and translocation of nuclear proteins
301	YLR249W	(YEF3)	3.59397167	3.14453335	2.63195398	Translation elongation factor EF-3A, member of ATP-binding cassette (ABC) superfamily
322	YOR337W	(TEA1)	2.13152473	2.97907151	4.82285812	TY1 enhancer activator of the GAL4P-type family of DNA-binding proteins
331	YCR084C	(TUP1)	2.40138822	2.92198431	3.27182635	General repressor of transcription (with SSN6P), member of WD (WD-40) repeat family
336	YPL226W	-	2.45263084	2.88856775	2.55579443	Protein with similarity to members of the ATP-binding cassette (ABC) superfamily
345	YJR019C	(TES1)	4.07777555	2.83032346	2.07248965	Acyl-CoA thioesterase
346	YBL008W	(HIR1)	7.24580603	2.82847131	2.88668127	Histone transcription inhibitor, required for periodic repression of 3 of the 4 histone gene loci and for autogenous repression of HTA1-HTB1 locus by H2A and H2B
349	YGL001C	-	3.91981575	2.82148161	1.98527852	Protein with similarity to nocardia SP. cholesterol dehydrogenase
359	YCL037C	(SRO9)	8.35007693	2.78557477	2.39358801	Suppressor of YPT6 null and RHO3 mutations
367	YDR088C	(SLU7)	2.07293165	2.73396273	2.68767436	Pre-mRNA splicing factor affecting 3' splice site choice, required only for the second catalytic step of splicing

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
376	YBR289W	(SNF5)	2.00671327	2.68812945	3.03861899	Component of SWI/SNF global transcription activator complex, acts to assist gene-specific activators through chromatin remodeling
400	YPL237W	(SUI3)	2.5966981	2.5628077	2.54794558	Translation initiation factor EIF2beta subunit
406	YOR127W	(RGA1)	3.85804733	2.53166489	2.56973414	RHO-type GTPase-activating protein (GAP) for CDC42P
416	YFR009W	(GCN20)	2.63782118	2.48595438	2.16133777	Component of a protein complex required for activation of GCN2P protein kinase in response to amino acid starvation, member of ATP-binding cassette (ABC) superfamily
417	YDR211W	(GCD6)	2.22567451	2.48354852	1.82406386	Translation initiation factor EIF2B (guanine nucleotide exchange factor), 81 KDA (beta) subunit
440	YGR270W	(YTA7)	2.68803581	2.4056715	1.94575504	Protein with similarity to members of the AAA family of ATPases
441	YBR119W	(MUD1)	2.83912216	2.40515252	1.39876418	U1 SNRNP A protein (SNRNA-associated protein) with 2 RNA recognition (RRM) domains
442	YDR052C	(DBF4)	6.85835185	2.40369283	1.58349756	Regulatory subunit for CDC7P protein kinase, required for G1/S transition
492	YPR080W	(TEF1)	2.50402057	2.21150946	1.85878786	Translation elongation factor EF-1alpha (TEF1 and TEF2 code for identical proteins)
496	YDR264C	(AKR1)	3.13151279	2.19839665	2.75165355	Ankyrin repeat-containing protein that has an inhibitory effect on signaling in the pheromone pathway
503	YDR422C	(SIP1)	2.62373247	2.18707608	2.08363472	Multicopy suppressor of SNF1, related to GAL83P/SPM1P and SPM2P
504	YMR080C	(NAM7)	2.82340116	2.1828046	2.17148277	Protein involved with NMD2P and UPF3P in decay of mRNA containing nonsense codons
515	YAL048C	-	5.02313141	2.13847476	4.22113197	Protein with weak similarity to RAS1P, RAS2P, and other GTP-binding proteins of the RAS superfamily
526	YHL027W	(RIM101)	2.57210755	2.10331571	2.59278915	Zinc-finger protein involved in induction of IME1
531	YGL112C	(TAF60)	2.21463331	2.07652653	2.18913076	Component of TAF(II) complex (TBP-associated protein complex) required for activated transcription by RNA polymerase II
549	YPL028W	(ERG10)	2.86559138	2.01859514	1.69893374	Acetyl-CoA acetyltransferase (acetoacetyl-CoA thiolase), first step in mevalonate/sterol pathway

Seq. Num.	Clone ID	ALIAS	CJ-4hr/ LP-4hr	K-50/CK	K-100/CK	Gene Description
621	YNR019W	(ARE2)	2.1	1.79103463	2.85442	Acyl-CoA:sterol acyltransferase (sterol-ester synthetase)
560	YFL054C	-	7.43223753	0.47935821	0.61365816	Protein with similarity to FPS1P and YPR192P, member of MIP family of transmembrane channels
562	YAL001C	(TFC3)	2.94740587	0.47427458	1.19155655	RNA polymerase transcription initiation factor TFIIC (TAU), 138 KDA subunit
579	YIR017C	(MET28)	2.97658904	0.3775372	0.30088953	Transcriptional activator of the basic leucine zipper (BZIP) family, works with MET4P and CBF1P to regulation sulfur amino acid metabolism
585	YDR253C	(MET32)	2.86314943	0.33971751	0.32790428	Zinc-finger protein involved in transcriptional regulation of methionine metabolism
595	YMR146C	(TIF34)	5.0351989	0.22482039	0.71935381	Translation initiation factor EIF3, P39 subunit, has 2 WD (WD-40) repeats
616	YGR083C	(GCD2)	2.32134339	0	0	Translation initiation factor EIF2B (guanine nucleotide exchange factor), 71 KDA (delta) subunit
610	YOR139C	(SFL1)	5.46648132	0	11.7609951	Transcription factor with domains homologous to MYC oncoprotein and yeast HSF1P, required for normal cell surface assembly and flocculence

\* Table Headings:

Clone ID: A clone ID designation number.

CJ-4hr/LP-4hr: Expression level in the mutant CJ517 as compared with the respective wild type strain LPY9 at 4hr sampling of log phase growth of yeast (ratio of mutant expression level/control expression level). Genes in the Table are either up or down regulated in the mutant (CJ517) as well as in response to ketoconazole treatment.

K-50/CK: Expression level in the wild type yeast LPY9, at 2 hr after treatment with 50micro gram/ml ketoconazole as compared to the wild type LPY9 strain without ketoconazole treatment (ratio of treatment expression level/control expression level).

K-100/CK: Expression level in the wild type yeast LPY9, at 2 hr after treatment with 100micro gram/ml ketoconazole as compared to the wild type LPY9 strain without ketoconazole treatment (ratio of treatment expression level/control expression level).

5 Gene Description: Description of the clone listed in column 1.

In addition, for example, Table 2 identifies a yeast HES1 gene as a gene with an associated change in mRNA levels in the two different comparisons. Fang *et al. EMBO J* 15:6447-59 (1996), the entirety of which is herein incorporated by reference, reported a mutation in *HES1*, which caused a 55% reduction in carbon flux through the mevalonate pathway in yeast.

10 Each of the sequences listed in Table 2 or 3 represents a gene that effects sterol levels, directly or indirectly, or whose expression changes as a result of alterations in the sterol synthesis pathway.

### Example 2

15 Sequences that encode for the yeast HES1 protein are used to search databases for homologues from other species. A number of different databases can be used for these searches, including, for example, dbEST, GenBank, EMBL, SwissProt, PIR, and GENES. In addition, various algorithms for searching can be selected, such as, for example, the BLAST suite of programs at the default values. Typically, matches found with BLAST P values equal or less than 0.001 (probability) or BLAST Score of equal or greater than 90 are classified as hits. If the  
20 program used to determine the hit is HMMSW then the score refers to HMMSW score. The GenBank database is searched with BLASTN and BLASTX (default values). Sequences that pass the hit probability threshold of  $10e^{-8}$  are considered hits.

**Table 4**

Seq. Num.	Clone ID	Sequence: DNA/Protein	Hit description	Library
1	701100307CPR9855	DNA	Yeast HES 1 homolog	SOYMON028
2	701001443CPR9857	DNA	Yeast HES 1 homolog	SOYMON018
3	701010572CPR9854	DNA	Yeast HES 1 homolog	SOYMON019
4	701176735CPR9736	DNA	Yeast HES 1 homolog	SATMONN05

Seq. Num.	Clone ID	Sequence: DNA/Protein	Hit description	Library
5	Z75145	DNA	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins	-
622	701100307CPR9855	Protein	Yeast HES 1 homolog	SOYMON028
623	701001443CPR9857	Protein	Yeast HES 1 homolog	SOYMON018
624	701010572CPR9854	Protein	Yeast HES 1 homolog	SOYMON019
625	701176735CPR9736	Protein	Yeast HES 1 homolog	SATMONN05
626	Z75145	Protein	Protein implicated in ergosterol biosynthesis, member of the KES1/HES1/OSH1/YKR003W family of oxysterol-binding (OSBP) proteins	-
6	701003888H1	DNA	Yeast HES 1 homolog	SOYMON019
7	701001351H1	DNA	Yeast HES 1 homolog	SOYMON018
8	700672545H1	DNA	Yeast HES 1 homolog	SOYMON006
9	700664054H1	DNA	Yeast HES 1 homolog	SOYMON005
10	700665644H1	DNA	Yeast HES 1 homolog	SOYMON005
11	700764248H1	DNA	Yeast HES 1 homolog	SOYMON020
12	700851444H1	DNA	Yeast HES 1 homolog	SOYMON023
13	700971910H1	DNA	Yeast HES 1 homolog	SOYMON005
14	700652932H1	DNA	Yeast HES 1 homolog	SOYMON003
15	700982894H1	DNA	Yeast HES 1 homolog	SOYMON009
16	701120140H1	DNA	Yeast HES 1 homolog	SOYMON037
17	701064234H1	DNA	Yeast HES 1 homolog	SOYMON034
18	700954013H1	DNA	Yeast HES 1 homolog	SOYMON022
19	701129375H1	DNA	Yeast HES 1 homolog	SOYMON037
20	701043941H1	DNA	Yeast HES 1 homolog	SOYMON032
21	LIB24-114-Q1-E1-H8	DNA	Arabidopsis HES 1 homolog	LIB24
22	LIB22-016-Q1-E1-F3	DNA	Arabidopsis HES 1 homolog	LIB22
23	LIB25-101-Q1-E1-F1	DNA	Arabidopsis HES 1 homolog	LIB25
24	AA042357	DNA	Arabidopsis HES 1 homolog	-
25	AA720163	DNA	Arabidopsis HES 1 homolog	-
26	Z29936	DNA	Arabidopsis HES 1 homolog	-
27	T76850	DNA	Arabidopsis HES 1 homolog	-
28	T76580	DNA	Arabidopsis HES 1 homolog	-
29	AA586043	DNA	Arabidopsis HES 1 homolog	-

Homologues to yeast HES1 are also identified in the following libraries: SOYMON003, SOYMON005, SOYMON006, SOYMON009, SOYMON018, SOYMON019, SOYMON020, SOYMON022, SOYMON028, SOYMON023, SOYMON032, SOYMON034, SOYMON027, SATMONN05, LIB22, LIB 24, and LIB 25. These libraries are prepared as follows:

- The SATMONN05 cDNA library is a normalized library generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign Illinois, U.S.A.) root tissue at the V6 development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three



times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in a greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The root system is cut from the mature maize plant and washed with water to free it from the soil. The tissue is immediately frozen in liquid nitrogen and the harvested tissue is then stored at -80°C until RNA preparation. The RNA is purified from the stored tissue. The library is normalized in two rounds using conditions adapted from Soares *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:9928 (1994), the entirety of which is herein incorporated by reference and Bonaldo *et al.*, *Genome Res.* 6: 791 (1996), the entirety of which is herein incorporated by reference except that a significantly longer (48 -hours/round) reannealing hybridization was used. SATMON003 is a root tissue library from the same donor.

The SOYMON003 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) hypocotyl axis tissue from seedlings 2 day after-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 2 days after the start of imbibition. The 2 days after imbibition samples are separated into 3 collections after removal of any adhering seed coat. At 2 days after imbibition under the above conditions, the seedlings have significant expansion of the axis and are close to emerging from the soil. A few seedlings have cracked the soil surface and exhibited slight greening of the exposed cotyledons. The seedlings are washed in water to remove soil, hypocotyl axis harvested and immediately frozen in liquid nitrogen. The harvested

tissue is then stored at  $-80^{\circ}\text{C}$  until RNA preparation. The RNA is purified from the stored tissue and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A<sup>+</sup> RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript<sup>TM</sup> Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON005 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) hypocotyl axis tissue from seeds 6 hour post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately  $29^{\circ}\text{C}$  and the nighttime temperature approximately  $24^{\circ}\text{C}$ . Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 6 hours after the start of imbibition. The 6 hours after imbibition sample is collected over the course of approximately 2 hours starting at 6 hours post imbibition. At the 6 hours after imbibition stage, not all cotyledons have become fully hydrated and germination. Radicle protrusion has not occurred. The seedlings are washed in water to remove soil, then the hypocotyl axis is harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at  $-80^{\circ}\text{C}$  until RNA preparation. The RNA is purified from the stored tissue and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the

manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

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The SOYMON006 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) cotyledons from seeds 6 hour post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 6 hours after imbibition. The 6 hours after imbibition sample is collected over the course of approximately 2 hours starting at 6 hours post-imbibition. At the 6 hours after imbibition, not all cotyledons have become fully hydrated and germination. Radicle protrusion has not occurred. The seedlings are washed in water to remove soil, then the cotyledon is harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation. The RNA is purified from the stored tissue and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON009 cDNA library is generated from soybean cutlivar C1944 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) pod and seed tissue harvested 15 days post-flowering. Pods from field grown plants are harvested 15 days post-flowering. The pods are picked from all over the plant, placed into 14ml polystyrene tubes and immediately immersed in dry-ice. Approximately 3g of pod tissue is harvested. The harvested tissue is then stored at – 80°C until RNA preparation. The RNA is purified from the stored tissue and the cDNA library is constructed. The RNA is purified from the stored tissue and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON018 cDNA is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) leaf tissue harvested from plants grown in a field in Jerseyville 45 and 55 days after flowering. Leaves from field grown plants are harvested 45 and 55 days after flowering from the fourth node. Approximately 27g and 33g of leaves are collected from the 45 and 55 days after flowering plants, placed into 14ml polystyrene tubes and

immediately immersed in dry ice. The harvested tissue is then stored at  $-80^{\circ}\text{C}$  until RNA preparation. Total RNA is prepared from the combination of equal amounts of leaf tissue from both time points and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON019 cDNA library is generated from soybean cultivars Cristalina (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and FT108 (Monsoy, Brazil) (tropical germ plasma) root tissue. Roots are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately  $29^{\circ}\text{C}$  and the nighttime temperature approximately  $24^{\circ}\text{C}$ . Soil is checked and watered daily to maintain even moisture conditions. Approximately 50g and 56g of roots are harvested from each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The plants are uprooted and the roots quickly rinsed in a pail of water. The root tissue is then cut from the plants, placed immediately in 14ml polystyrene tubes and immersed in dry-ice. The harvested tissue is then stored at  $-80^{\circ}\text{C}$  until RNA preparation. Total RNA is prepared from the combination of equal amounts of root tissue from each cultivar and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as

recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The  
5 Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON020 cDNA is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seeds harvested from plants grown in a field in  
10 Jerseyville 65 and 75 days post-flowering. The seed pods are picked from all over the plant and the seeds extracted from the pods. Approximately 14g and 31g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at –80°C until RNA preparation. Total RNA is prepared from the combination of equal numbers of seeds from 65 and 75 days after flowering and the cDNA library is constructed.

15 The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

20 Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

25 The SOYMON022 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) partially to fully opened flower tissue, which is harvested from plants grown in an environmental chamber. Seeds are planted in moist

Metromix 350 medium at a depth of approximately 2cm. Trays are placed in an environmental chamber set to a 12h day/12h night cycle, 29°C daytime temperature, 24°C night temperature and 70% relative humidity. Daytime light levels are measured at 450μEinsteins/m<sup>2</sup>. Soil is checked and watered daily to maintain even moisture conditions. Flowers are removed from the plant at the pedicel. Flower buds showing petal color to fully open flowers are selected for collection. A total of 3g of flower tissue is harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation. Total RNA is prepared from a mixture of opened and partially opened flowers and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON023 cDNA library is generated from soybean genotype BW211S Null (Tohoku University, Morioka, Japan) seed tissue harvested from plants grown in a field in Jerseyville. After 15 and 40 days, pods are harvested from all over the plant and seeds are dissected out from the pods. Approximately, 0.7g and 14.2g of seeds are harvested from the plants at the 15 and 40 days after flowering timepoints. The seeds are placed into 14ml polystyrene tubes and immersed in dry-ice. The tissue is then transferred to a -80°C freezer for storage. The harvested tissue is then stored at -80°C until RNA preparation. Total RNA is prepared from the combination of 0.5g and 1.0g of seeds from the 15 and 40 days after flowering timepoints and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON028 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought-stressed root tissue. Seeds are planted in moist Metromix 350 medium at a depth of approximately 2cm in trays. The trays are placed in an environmental chamber set to a 12h day/12h night cycle, 26°C daytime temperature, 21°C night temperature and 70% relative humidity. Daytime light levels are measured at 300μEinsteins/m<sup>2</sup>. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of development, water is withheld from half of the plant collection (drought stressed population). After 3 days, half of the plants from the drought stressed condition and half of the plants from the control population are harvested. After another 3 days (6 days post drought induction) the remaining plants are harvested. A total of 27g and 40g of root tissue is harvested from plants at two time points and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation. Total RNA is prepared from the combination of equal amounts of drought stressed root tissue from both time points and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as



recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The  
5 Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON032 cDNA library is prepared from the Asgrow cultivar A4922 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) rehydrated dry soybean seed meristem tissue. Surface  
10 sterilized seeds are germinated in liquid media for 24 hours. The seed axis is then excised from the barely germinating seed, placed on tissue culture media and incubated overnight at 20°C in the dark. The supportive tissue is removed from the explant prior to harvest. Approximately 570mg of tissue is harvested and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation. The RNA is purified from the stored tissue and the cDNA library  
15 is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York  
20 U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the  
25 manufacturer.

The SOYMON034 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) cold-shocked seedling tissue without

cotyledons. Seeds are imbibed and germinated in vermiculite for 2 days under constant illumination (*ca.* 510 Lux). After 48 hours, the seedlings are transferred to a cold room set at 5°C under constant illumination (*ca.* 560 Lux). After 30, 60 and 180 minutes seedlings are harvested and dissected. The seedlings after 2 days of imbibition are beginning to emerge from the  
5 vermiculite surface. The apical hooks are dark green in appearance. A portion of the seedling consisting of the root, hypocotyl and apical hook is frozen in liquid nitrogen and stored at -80°C. Total RNA is prepared from equal amounts of pooled tissue and the cDNA library is constructed.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the  
10 manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The  
15 Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The SOYMON037 cDNA library is generated from soybean cultivar A3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) etiolated axis and radical tissue. Seeds are planted in  
20 moist vermiculite, wrapped and kept at room temperature in complete darkness until harvest. Etiolated axis and hypocotyl tissue is harvested at 2, 3 and 4 days post-planting. Samples are frozen in liquid nitrogen upon harvesting and stored at -80°C until RNA preparation. 1 gram of each sample (axis + hypocotyl at day 2, 3 and 4) is pooled for RNA isolation. The RNA is purified from the pooled tissue and the cDNA library is constructed.

25 The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as

recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The  
5 Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The cDNA library of the present invention designated LIB22, is prepared from  
*Arabidopsis thaliana* Columbia ecotype root tissue. Wild type *Arabidopsis thaliana* seeds are  
10 planted in commonly used planting pots and grown in an environmental chamber. After 5-6 weeks the plants are in the reproductive growth phase. Stems are bolting from the base of the plants. After 7 weeks, more stems and floral buds appear, and a few flowers are starting to open. Roots of 7-week old plants from pots are rinsed intensively with tap water to wash away dirt, and briefly blotted by paper towel to take away free water. The tissues are immediately frozen in  
15 liquid nitrogen and stored at -80°C until total RNA extraction.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York  
20 U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the  
25 manufacturer.

The cDNA library of the present invention designated LIB24, is prepared from  
*Arabidopsis thaliana*, Columbia ecotype, flower bud tissue. Wild type *Arabidopsis thaliana*

seeds are planted in commonly used planting pots and grown in an environmental chamber. Flower buds are green and unopened and are harvested about seven weeks after planting. The tissue is immediately frozen in liquid nitrogen and stored at -80°C until total RNA extraction.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

The cDNA library of the present invention designated LIB25, is prepared from *Arabidopsis thaliana*, Columbia ecotype, open flower tissue. Wild type *Arabidopsis thaliana* seeds are planted in commonly used planting pots and grown in an environmental chamber. Flower are completely opened with all parts of floral structure observable, but no siliques are appearing, and are harvested about seven weeks after planting. The tissue was immediately frozen in liquid nitrogen and stored at -80°C until total RNA extraction.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life

Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

### Example 3

#### Detection of Changes in Sterol Metabolism

5 A labeled acetyl-CoA molecule, squalene molecule, or acetate are used in a variety of assays to detect changes in sterol production, secretion, localization, protein-binding, degradation, and trafficking known in the art. The example below illustrates.

Cells from transformed plants are cultured in an appropriate medium. Labeled acetate, preferably  $^{14}\text{C}$ -labeled, is added to a concentration of about 1 uCi/ml. After a period of growth,  
10 the cells are collected, the lipids extracted, and resolved by thin-layer chromatography or run over HPLC column using known methods. The levels of each sterol resolved can be compared to control cells fed the same labeled  $^{14}\text{C}$  acetate and the amount of  $^{14}\text{C}$ -labeled sterol for each determined from the resolved sterols.

#### References

15 In addition to those references cited and incorporated by reference above, the below references are incorporated in their entirety. In addition, these references, as well as each of those cited in the Summary and Detailed Description above, can be relied upon to make and use aspects of the invention.

Jiang, *et al.*, A new family of yeast genes implicated in ergosterol synthesis is related to the human oxysterol-binding protein. *Yeast* 10: 341-53 (1994).

Fang, *et al.*, Kes1p shares homology with human oxysterol-binding protein and participates in a novel regulatory pathway for yeast Golgi-derived transport vesicle biogenesis. *EMBO J.* 15: 6447-59(1996).

Crowley, *et al.*, A mutation in a purported regulatory gene affects control of sterol uptake in *Saccharomyces cerevisiae*. *Journal of Bacteriology*, 180(16): 4177-83 (1998).

25 Casperand Holt. Expression of the green fluorescent protein-encoding gene from a tobacco mosaic virus-based vector. *Gene* 173: 69-73 (1996).

**We claim:**

1. A substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 622.
- 5 2. The substantially purified nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 1.
3. A substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 1 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 622.
- 10 4. The substantially purified nucleic acid molecule according to claim 3, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 1 or its complement under high stringency conditions.
- 15 5. The substantially purified nucleic acid molecule according to claim 3, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 1 or its complement under low stringency conditions.
6. A substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 623.
7. The substantially purified nucleic acid molecule of claim 6, wherein the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 2.
- 20 8. A substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 2 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 623.
- 25 9. The substantially purified nucleic acid molecule according to claim 8, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 2 or its complement under high stringency conditions.

10. The substantially purified nucleic acid molecule according to claim 8, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 2 or its complement under low stringency conditions.

11. A substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 624.

12. The substantially purified nucleic acid molecule of claim 11, wherein the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 3.

13. A substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 3 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 624.

14. The substantially purified nucleic acid molecule according to claim 13, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 3 or its complement under high stringency conditions.

15. The substantially purified nucleic acid molecule according to claim 13, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 3 or its complement under low stringency conditions.

16. A substantially purified nucleic acid molecule that encodes a protein comprising the amino acid sequence of SEQ ID NO: 625.

17. The substantially purified nucleic acid molecule of claim 16, wherein the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 4.

18. A substantially purified nucleic acid molecule that specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 4 or its complement, wherein the nucleic acid molecule encodes a protein comprising the amino acid sequence of SEQ ID NO: 625.

19. The substantially purified nucleic acid molecule according to claim 18, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 4 or its complement under high stringency conditions.

20. The substantially purified nucleic acid molecule according to claim 18, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid sequence of SEQ ID NO: 4 or its complement under low stringency conditions.

21. A substantially purified nucleic acid molecule comprising a nucleic acid sequence which encodes a plant HES1 protein.

22. A substantially purified protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 622, 623, 624, and 625.

23. An antibody capable of specifically binding a protein with the amino acid sequence of SEQ ID NO: 622.

24. An antibody capable of specifically binding a protein with the amino acid sequence of SEQ ID NO: 625.

25. A transformed plant having a nucleic acid molecule which comprises:

- (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to
- (B) a structural nucleic acid molecule, wherein said structural nucleic acid molecule comprises a nucleic acid sequence encoding a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 622 through SEQ ID NO: 626 or fragment thereof; which is linked to
- (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

26. The transformed plant according to claim 25, wherein said structural gene is in the antisense orientation.

27. The transformed plant according to claim 25, wherein said plant is selected from the group of rapeseed, maize, soybean, safflower, sunflower, cotton, peanut, flax, oil palm and Cuphea.



### Abstract

This invention relates to the field of biotechnology, particularly as it pertains to the production of sterols in a variety of host systems particularly plants. More specifically, the invention relates to nucleic acid molecules encoding proteins and fragments of proteins

5 associated with sterol and phytosterol synthesis and metabolism as well as the encoded proteins and fragments of proteins and antibodies capable of binding to them. The invention also relates to methods of using the nucleic acid molecules, fragments of the nucleic acid molecules, proteins, and fragments of proteins. The invention also relates to cells, organisms, plants, or seeds, or progeny of any, that have been manipulated to contain increased levels or overexpress  
10 at least one sterol or phytosterol compound.

<110> Karunanandaa, Balasulojini  
Yu, Jaehyuk  
Kishore, Ganesh M.

<120> NUCLEIC ACID MOLECULES AND OTHER MOLECULES ASSOCIATED  
WITH STEROL SYNTHESIS AND METABOLISM

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Thr Ala Leu His Ala Thr Asp Glu Lys Glu Asn Ile Glu Met Leu Trp		
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Cys Gln Arg Pro Asp Pro Lys Phe Asn Gly Thr Ser Val Glu Ala Lys	
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Val His Gly Ile Arg Gln Leu Lys Leu Leu Asn His Gly Glu Thr Tyr	
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Glu Met Asn Cys Pro Arg Leu Leu Leu Arg Ile Leu Pro Val Pro Gly	
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Val Ala Glu Leu Ser Tyr Arg Ser Ser Ser Phe Leu Gly Ile Gly Gly	
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Val Leu Tyr Glu Val Asp Gly His Trp Asp Arg Thr Val Lys Val Lys	
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Asp Thr Asn Asn Gly Lys Val Arg Val Ile Tyr Asp Ala Lys Glu Val	
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Gln Thr Glu Ser Ala His Val Trp Gly Glu Leu Asn Gln Ala Ile Val	
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Ser Lys Asp Trp Glu Lys Ala Arg Glu Ala Lys Leu Lys Val Glu Glu	
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Arg Gln Arg Glu Leu Val Arg Glu Arg Glu Ser Lys Gly Glu Thr Trp	
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Ile Ser Lys His Phe Val Val Ser Asn Asn Lys Glu Gly Trp Gln Cys	
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Ser Pro Ile His Lys Ser Val Pro Ala Ala Pro Ile Thr Ala Leu	

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 Leu Val Tyr Ala Ser Ser Phe Phe Ile Ser Val Tyr Tyr Ala Tyr Gln  
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 Arg Thr Trp Lys Pro Phe Asn Pro Ile Leu Gly Glu Thr Tyr Glu Met  
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 Val Asn His Gly Gly Ile Thr Phe Ile Ser Glu Gln Val Ser His His  
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 Pro Pro Met Ser Ala Gly His Ala Glu Thr Glu His Phe Thr Tyr Asp  
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 Val Thr Ser Lys Leu Lys Thr Lys Phe Leu Gly Asn Ser Val Asp Val  
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 Tyr Pro Val Gly Arg Thr Arg Val Thr Leu Lys Arg Asp Gly Val Val  
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Arg Thr Trp Ile Asp Ser Pro Gly Glu Met Ile Leu Thr Asn Leu Thr				
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Thr Gly Asp Lys Val Val Leu Tyr Phe Gln Pro Cys Gly Trp Phe Gly				
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Ala Gly Arg Tyr Glu Val Asp Gly Tyr Val Tyr Asn Ser Ala Asp Glu				
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Pro Lys Ile Leu Met Thr Gly Lys Trp Asn Glu Ala Met Asn Tyr Gln				
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Val Cys Asp Ser Glu Gly Glu Pro Leu Pro Gly Thr Glu Leu Lys Glu				
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Ile Trp Arg Val Ala Asp Thr Pro Lys Lys Asp Lys Phe Gln Tyr Thr				
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His Phe Ala His Lys Ile Asn Ser Phe Asp Thr Ala Pro Lys Lys Leu				
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Leu Ala Ser Asp Ser Arg Leu Arg Pro Asp Arg Met Ala Leu Glu Lys				
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Gly Asp Leu Ser Thr Ser Gly Tyr Glu Lys Ser Ser Leu Glu Glu Arg				
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Gln Arg Ala Glu Lys Arg Asn Arg Glu Ala Lys Gly His Lys Phe Thr				
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Asp Leu Glu Val Tyr Gln Tyr Asn Gly Lys Tyr Thr Gln His Cys Ala				
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gcc gtt gat agt tct gag tgc att gaa gtg cct gac atc aga cca gaa				1060
Ala Val Asp Ser Ser Glu Cys Ile Glu Val Pro Asp Ile Arg Pro Glu				
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Ser Trp Ser Ser Phe Leu Lys Ser Ile Ala Ser Phe Asn Gly Asp Leu
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tcc tct ctc acc gca ccg ccg ttc atc ctc tca aca acc tct tta acc      144
Ser Ser Leu Thr Ala Pro Pro Phe Ile Leu Ser Thr Thr Ser Leu Thr
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gag tat tct gcg tac tgg tgc gaa cat cct gca ctc ttc gtt gcc ccc      192
Glu Tyr Ser Ala Tyr Trp Cys Glu His Pro Ala Leu Phe Val Ala Pro
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gca cgt gag ccc gat cct gcg aag aga gcg ctc ttg gtg ctg aaa tgg      240
Ala Arg Glu Pro Asp Pro Ala Lys Arg Ala Leu Leu Val Leu Lys Trp
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ttc ctg agc aca ttg cac caa cag tac tgc tct cga agc gaa aag cta      288
Phe Leu Ser Thr Leu His Gln Gln Tyr Cys Ser Arg Ser Glu Lys Leu
    85                90                95

gga agc gag aaa aag ccg ctc aac ccg ttc ctg ggc gag ctt ttc ctg      336
Gly Ser Glu Lys Lys Pro Leu Asn Pro Phe Leu Gly Glu Leu Phe Leu
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Glu Gln Val Ser His His Pro Pro Ala Thr Ala Tyr Ser Ile Val Asn	
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Glu Lys His Gly Val Glu Leu Gln Gly Tyr Asn Ala Gln Lys Ala Ser	
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Phe Ser Ser Thr Ile Gln Val Lys Gln Leu Gly His Ala Tyr Leu Ser	
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Leu Thr Pro Pro Gly Lys Asp Ala Asn Asn Glu Asp Asp Arg Glu His	
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Tyr Leu Ile Thr Leu Pro Asn Leu His Ile Glu Ser Leu Ile Tyr Gly	
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Thr Pro Phe Val Glu Leu Glu Lys Ser Cys Lys Ile Ala Ser Ser Thr	
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Gly Tyr Ile Ser Lys Ile Asp Phe Ser Gly Lys Gly Trp Leu Ser Gly	
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Ile Arg Asp Ala Arg Ala Lys Lys Asp Ile Glu Thr Phe Thr Ile Ser	
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Asn Leu Lys Thr Thr Pro Leu Thr Val Ala Pro Leu Asp Glu Gln Asp	
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Arg Gly Asp Met Glu Ala Thr Ser Asn Ala Lys Thr Lys Ile Glu Val	

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Ala Gln Arg Glu Leu Arg Lys Lys Glu Lys Glu Gln Gly Glu Glu Trp				
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gaa cga cga ttc ttc aag cga gtc aac gaa aag gat gaa cct acc ttt				1104
Glu Arg Arg Phe Phe Lys Arg Val Asn Glu Lys Asp Glu Pro Thr Phe				
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atg aga ttg gcg gcg atg ctg gat ttg acg caa ggc atc gaa agt gac				1152
Met Arg Leu Ala Ala Met Leu Asp Leu Thr Gln Gly Ile Glu Ser Asp				
	370	375	380	
cgc acc ggg gga gtt tgg agg ttt gat cct tca cgt gct gtg gat gcg				1200
Arg Thr Gly Gly Val Trp Arg Phe Asp Pro Ser Arg Ala Val Asp Ala				
	385	390	395	400
aat ccg ccg tat cac aag gtt ggc ggc gaa ggg ttg gga ttg taa				1245
Asn Pro Pro Tyr His Lys Val Gly Gly Glu Gly Leu Gly Leu				
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Met Ser Gln His Ala Ser Ser	
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Ser Ser Trp Thr Ser Phe Leu Lys Ser Ile Ser Ser Phe Asn Gly Asp	
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Leu Ser Ser Leu Ser Ala Pro Pro Phe Ile Leu Ser Pro Thr Ser Leu	
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Thr Glu Phe Ser Gln Tyr Trp Ala Glu His Pro Ala Leu Phe Leu Glu	
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Pro Ser Leu Ile Asp Gly Glu Asn Tyr Lys Asp His Cys Pro Phe Asp	
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Pro Asn Val Glu Ser Lys Glu Val Ala Gln Met Leu Ala Val Val Arg	
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Trp Phe Ile Ser Thr Leu Arg Ser Gln Tyr Cys Ser Arg Ser Glu Ser	
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Met Gly Ser Glu Lys Lys Pro Leu Asn Pro Phe Leu Gly Glu Val Phe	
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Leu Leu Ser Glu Gln Val Ser His His Pro Pro Met Thr Ala Phe Ser	
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Ile Phe Asn Glu Lys Asn Asp Val Ser Val Gln Gly Tyr Asn Gln Ile	
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Lys Thr Gly Phe Thr Lys Thr Leu Thr Leu Thr Val Lys Pro Tyr Gly	
170 175 180	
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His Val Ile Leu Lys Ile Lys Asp Glu Thr Tyr Leu Ile Thr Thr Pro	
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Pro Leu His Ile Glu Gly Ile Leu Val Ala Ser Pro Phe Val Glu Leu	

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Gly Gly Arg Ser Phe Ile Gln Ser Ser Asn Gly Met Leu Cys Val Ile	220	225	230	
gaa ttt tca gga agg ggg tat ttc aca ggg aag aag aac tcc ttt aag				1193
Glu Phe Ser Gly Arg Gly Tyr Phe Thr Gly Lys Lys Asn Ser Phe Lys	235	240	245	
gca aga att tac aga agc cca caa gag cat agt cat aaa gaa aat gcg				1241
Ala Arg Ile Tyr Arg Ser Pro Gln Glu His Ser His Lys Glu Asn Ala	250	255	260	
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Leu Tyr Leu Ile Ser Gly Gln Trp Ser Gly Val Ser Thr Ile Ile Lys	265	270	275	
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Lys Asp Ser Gln Val Ser His Gln Phe Tyr Asp Ser Ser Glu Thr Pro	280	285	290	295
act gaa cat tta tta gtt aag cca atc gaa gaa caa cat cct ctg gaa				1385
Thr Glu His Leu Leu Val Lys Pro Ile Glu Glu Gln His Pro Leu Glu	300	305	310	
agt agg agg gca tgg aag gat gtg gca gaa gca atc aga caa gga aat				1433
Ser Arg Arg Ala Trp Lys Asp Val Ala Glu Ala Ile Arg Gln Gly Asn	315	320	325	
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Ile Ser Met Ile Lys Lys Thr Lys Glu Glu Leu Glu Asn Lys Gln Arg	330	335	340	
gcc ttg aga gaa caa gaa cgc gta aaa ggt gtg gaa tgg caa aga aga				1529
Ala Leu Arg Glu Gln Glu Arg Val Lys Gly Val Glu Trp Gln Arg Arg	345	350	355	
tgg ttc aaa caa gtg gac tac atg aat gaa aat aca tca aat gat gta				1577
Trp Phe Lys Gln Val Asp Tyr Met Asn Glu Asn Thr Ser Asn Asp Val	360	365	370	375
gag aaa gca agt gaa gat gat gcc ttt agg aaa ttg gcg tcc aaa ctg				1625
Glu Lys Ala Ser Glu Asp Asp Ala Phe Arg Lys Leu Ala Ser Lys Leu	380	385	390	
cag ctt tct gtg aaa aat gtg cca agt ggg aca ttg att ggc ggc aaa				1673
Gln Leu Ser Val Lys Asn Val Pro Ser Gly Thr Leu Ile Gly Gly Lys	395	400	405	
gat gat aag aaa gat gtt tca acc gca ttg cat tgg agg ttt gat aaa				1721
Asp Asp Lys Lys Asp Val Ser Thr Ala Leu His Trp Arg Phe Asp Lys	410	415	420	

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agtcaaccct ccagtatctg ccctccatgc aacagatgag anggaaaaca ttgagatgat 60  
 atgggtcccag caacctgttc caaagtttcg ggggtacatct atgaagctca agtgcattggt 120  
 aaacgtcata tgtttctcca tgatttagga gcttcagctg acgtttacca tgcacttgag 180  
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 attcttccgg ttctctggga 259

<210> 14  
 <211> 355  
 <212> DNA  
 <213> Glycine max

<220>  
 <223> unsure at all n locations

<400> 14

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 gaaaagggtta gtcattcatcc aatgattgtt gcttgctact gtgaggggaag gggatggaag 180  
 ttttgggcag attctaattt gaaaggaaaa ttctgggggc gttctatcca gttagatcct 240  
 gtgggtgtcc tcaactctaca gtttgaggat ggtgaaacat ttcagtggag caaggtcacc 300  
 acttcgattt acaatatcat actangtaaa atttattgtg accactacgg tacca 355

<210> 15  
 <211> 279  
 <212> DNA  
 <213> Glycine max

<220>  
 <223> unsure at all n locations

<400> 15

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 ggaagatgat gcagaagtat attggctcgg atgtaacatc aatgggtgaca ctaccagtta 120  
 ttatatattga accaatgact atgattcaga aaattgctga gttgatggag tactcctact 180  
 tgttagatca agcagatgaa tcagaggatc catacatgca gttagtttat gcaatggatg 240  
 tactttnatgt atcatcacag catccatggg ccatatcgg 279



<210> 16  
 <211> 191  
 <212> DNA  
 <213> Glycine max  
  
 <400> 16  
  
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 tatgataatt tggatgctga ataataagca tccttgtaga attctttcta ttctttgaac 120  
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 ttttgggtaa a 191

<210> 17  
 <211> 267  
 <212> DNA  
 <213> Glycine max  
  
 <220>  
 <223> unsure at all n locations  
  
 <400> 17  
  
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 ccatggcagt atggtaattt ggccacggaa tgaactagtt tcaatttctt tggttttgga 180  
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 ncnnanngtt agttgggcng tgtacgc 267

<210> 18  
 <211> 252  
 <212> DNA  
 <213> Glycine max  
  
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 tcaatgctat ggtgaatcag tgtactgcac atcttcaaac ttgctgagca aatgcaacaa 180  
 tgggcagagt ccaactggaca ggttcacatc agtagtagca tggagcatat ctaccacacg 240  
 cccacatct tt 252

<210> 19  
 <211> 241  
 <212> DNA  
 <213> Glycine max

<400> 19

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 agaacgcgtg ttaccctcaa aagagatggg gtggctcctg atttggtgcc tcctcctaca 180  
 aaagttagca acttgatttt tggacgaact tggattgatt caccaggaga gatgatcctg 240  
 a 241

<210> 20  
 <211> 262  
 <212> DNA  
 <213> Glycine max

<400> 20

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 aagccattat gaacaaagat tgggaaagag caagagaagc aaagcaagac gtggaagaaa 180  
 gacagaggaa tatgttgaga gacagagcca tgacaggaga aactggttgt ctaagaattt 240  
 aggggtgtctt acagtaaaga ca 262

<210> 21  
 <211> 463  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <223> unsure at all n locations

<400> 21

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 aacgacaact ttcagtacac tcactttgct cacaagataa acagcttcga cacagcgcc 120  
 gctaagctct tggcttcaga ctcacgtatc cgctcctgata gatattccct tgagcagggt 180

gacctttcta aagctgggtc cgagaaacac agccttgagg agagacaaag ggccgaaaag 240  
aggaccagag agacaaaggg acaaaagttc actccaagat ggttcgatct aacggatgag 300  
atcacaccta ctccatgggg agatattgaa gtataccant acaacgggaa gtacaatgaa 360  
caccgagaca cggcagagag ctcaagtagt gcctccaacg aaacgggact caaatccatc 420  
gagtttaatc cttggcaata tggtaatatc tcaaccgaat gaa 463

<210> 22  
<211> 399  
<212> DNA  
<213> Arabidopsis thaliana

<400> 22

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gatgacaaat atcaatacac tcactttgct cacaagatta atagcttcga cactgccccg 120  
aaaaagctgt tgccctctga ttcacggtta cgacctgata gatacgact tgagatgggc 180  
gacatgtcca aatcaggcta tgagaagagc agcatggaag agagacagag agctgacaag 240  
agaacccgcg aacataaagg ccaagccttt actccaaaat ggttcgatgt aacggaagaa 300  
gtcactgcta caccatgggg tgatctggaa gtttaccat tcaactggaaa gtactcagaa 360  
catcgtgcag ctgcggtata ctctgaagat aagaccgac 399

<210> 23  
<211> 343  
<212> DNA  
<213> Arabidopsis thaliana

<400> 23

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tctgcttggg gaatgcagcc gccgtgatct tcccattgaa cggctcaaat cagtgggtgac 120  
gtggaacatc tccacactcc gtccgggtgt ctttggcatg tctccgtaca actccgttct 180  
cggcgagact caccacgtat cgaacgggtca catcaacgtc atcgccgaac aagtagtgca 240  
tcacctctcg gtttccgctc ttcatgcgac tcacgaacaa gaaaatatcg acgtgacatg 300  
gtgtcaatat ttcactccta aatttcgtgg tactcacgtg gac 343

<210> 24

<211> 510  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <223> unsure at all n locations

<400> 24

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 tggaattgcc atggggttgaa ttggatcgac ttagggtcgg tgttatcttc agagttatcc 180  
 gcagctgcac gatgttccga gtactttcca ttgaattggg aaacttccag atcaccccat 240  
 ggtgtagcag tgacttcttc cgttacatcg aaccattttg gagtaaaggc ttggcctttc 300  
 tcttcgcggg gtcncttttc aagtctctgt cncctttcca tgggtgntctt cccanagcct 360  
 gatttgnaca tggcgggcan cccaaggng gatcaatcag gccgnaacgg ggaatcagnn 420  
 ggnaacagct tttcngggna ntgncgaagc aataaacnt gggggcaaag gggggggatt 480  
 ggaaattggc aacccttggg naacaggggc 510

<210> 25  
 <211> 282  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <223> unsure at all n locations

<400> 25

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 cctacaccat ggggtgatct cgaagtttac caattcantg gaaagtactc ggtgcaccgn 180  
 gccacagctg aaaactntga ggatacaacc gntgtgaagt tgncccaatt caacccttgg 240  
 caattccaag atctctntgc ttaatccttt ggtgccattt gt 282

<210> 26  
 <211> 380  
 <212> DNA  
 <213> Arabidopsis thaliana



aaaagtctgt cttcctgttt acttcaacga gccactttct tctttacaga aatgttttga 120  
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 cctcatgagg atacttaatg tagctgcttt tgctgtatct ggggatgcat caactgaagg 240  
 aagaatttgc aaacctttta atccattggt aggtgaaaca tacgnngcag actatccaga 300  
 caaaggcctt cggttttttt ccaggaaagg tcagtcatca tcctatgggt gtcgnatgcc 360  
 attgtgatgg caccnggtgg gaattcttgg gggacagcaa tcttnggggc aaattttggg 420  
 gcgntctntt tagcttnacc cccttgggga ttnnccttna aattnatgat ggggaanccn 480  
 caggggggaa ggngcccacc atnncaaacc 510

<210> 29  
 <211> 493  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <223> unsure at all n locations

<400> 29

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 ncgnaggga gccagcctt tctcccaaat ggtcgatgta ccggaggaag tcaactgtac 180  
 cccatggggt gatctggaag tttcccaatt caatggaaag tactcggaac atcgtgcagc 240  
 tgcggataac tctgaagata acaccgacct taagtcgatc caattcaacc catggcaatt 300  
 ccaagatctg tctacttaaa tgtatcgctc caaaagacag aaaagatcaa atcttttttg 360  
 aaacaaatgt attcttattc tctcgtagtt acaaaaaact ttgttctaca tctgctagct 420  
 ttcccatgac tttctctagt attagtgtac aacttctact gttttgtctt aaattcattc 480  
 aaatctttct ttg 493

<210> 30  
 <211> 1305  
 <212> DNA  
 <213> Saccharomyces cerevisiae

<400> 30

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 aacggagatc tatcgtcttt gtctgcacca ccgtttattc tttctccac ttccttaaca 120  
 gagttttctc agtattgggc tgaacatcca gctttatttc tggagccttc gttgattgat 180  
 ggtgaaaact acaaagatca ctgtcccttt gacccaaatg tggaatcaaa ggaagtggcg 240  
 cagatgttgg cggttgttag gtggtttatt tctactttga gatctcaata ctgctctaga 300  
 agcgaatcga tgggttctga aaagaagcct ttgaacccat tcttgggtga ggtatttgtt 360  
 ggaaagtga aaaatgatga gcatccagag tttggtgaaa cggttctttt aagtgaagcaa 420  
 gtttcacatc atccacctat gacagcattt tcgattttta atgaaaaaaaa tgatgtttct 480  
 gttcaaggat acaatcaa ataaaactgg tttacaaaaa cattgacgct aacggtcaaa 540  
 ccatacgggc atgtcatttt gaagattaaa gatgagacct acctgattac aacccgcct 600  
 ttgcatatcg aaggtatttt agtcgcttct ccatttgttg aattaggagg caggtcattc 660  
 atacagtcac caaatgggtat gttatgtgtt atagaatttt caggaagggg gtatttcaca 720  
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 gttaagccaa tcgaagaaca acatcctctg gaaagtagga gggcatggaa ggatgtggca 960  
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<210> 31  
 <211> 1200  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 31

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ttcaactctc cccaaagttt tccacatact gatataccat cagaagaagg taccaaagtt 300  
attctataa 309

<210> 33  
<211> 4014  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
  
<400> 33

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 gacaagtggg atgattgtaa accaaatata agtttagagc ataacgttcc gatcatcaga 2340  
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<210> 35  
 <211> 303  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 35  
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 tcaccaccag caggcgtcag tagatcactg cgcacatg taatgtggaa gcagagtaaa 180  
 ttaactcccc caagatttgt gaagatcatg aatagacgcc ctctgttcac agaaactagt 240  
 cagcctgtg cgaagtgcc aaaaacgtca caattactaa agtatgttta cacaaaagct 300  
 taa 303

<210> 36  
 <211> 888  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 36  
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 ccaaccactc tattgagcac gccagtacgt ctcaaaaatg gctttggaac accatcgccg 180  
 ccgtcaccac caggcataac gaaaagcatc actaaatcga gaagaaggcc gtcgacaacg 240

agtcttcagg gtatattcat gtcgcccgtc aataagcgtc gtgtcggcat aaccgcacat 300  
 ggacgtgtat atgaccataa cgacgacgga cacgaaagt agagtgagga cgacgaaaat 360  
 gaagaagaaa atgaaaatca aaagaagtac gacggacacg ttagtatgcc tttgttgcca 420  
 ccgacaacgc ccaagtcaag acggtcggag gtgttttctgt cgccgtcgcc gcgtttgagg 480  
 tcacctccga cggctgcgcg tcggtctacg ggcgagcggc ccatccgcga aatatcacat 540  
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 gcgctcggag gccaccacc acgggaggag gccacggctg tcgaaacact gatgctattg 780  
 tcgtcgcta ccaagaagca acaacaccga cccgtgccgg cgacatctgc tggagaaccc 840  
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<210> 37  
 <211> 2121  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 37

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 ctacatttaa ttactgtgga gaatattaag cctgaagaaa tacttatatt ttcgttaaca 180  
 aacaaagcag ttgatagtat aatagaaaat ttactgtcca ttttcgaaaa ctgcataca 240  
 aataaggaaa ttgtccatca gattgggtgt tacacagttc atgggttggc taatagaatc 300  
 gttgtagaga atgaggggat gataaacatt atagaagaga ttggatggag aggattaatg 360  
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 aaagttgtta aggattacaa gctaaacaac gcaaaaaata ataaccccg cattgagaaa 480  
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 aaaaaatact tagagcttga ttcttctgat tcagatgcat cgtcttttac tcaagatctc 600  
 cgcaataaat acaaggttgt tcttatcgat gaatttcagg acctctatcc aagtttagca 660  
 cccttaatta ccatgatttg caaaggcaag cagctcatta tgtttgggtga tacaatcaa 720

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 atctctctag catcaaaaat tatcaatcgt ccactagcag agaaacagat tatcgatgat 900  
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 aaggtctcaa aatctaataa gccccgggt atttcatttg ttaaattggt tctagagaca 1680  
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 gggacagtaa aactatctac catccattct gcaaagggtt tagaatttcc catagtattt 1800  
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 gatttgaaac gatctgtatg tgacgttaag gtgacacatg gttataatgt gcagcgatac 2040  
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 agcgttttca gacgtatata g 2121

<210> 38  
 <211> 3414  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400>

38

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gagaaaaatg tacctagatc aaacggaaga accaaaaacg aacacaatag tatggatgac 180  
gaagagtttg aattcttcca tcaatttagt cgggagaaag tttaaaggcgt catacacgta 240  
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ccggaacaga cgaacgacaa attgctgaca ttactaaatc agctattccc actaggaaac 360  
ggtcaaccag tcaacgaaaa aaaacagctc agaattgttt ccaaggccga tgtctggact 420  
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 <213> *Saccharomyces cerevisiae*

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gatgattttg accggggagtt tatgtggaac tcgtttctta tggacgaaat catcacatac 660  
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accattatth ccaggcaaag ttggaaaaga gcaggcacia ggtttaatgc acgtgggtata 840  
gatgatgatg gccatgtcgc aaattttgtt gaaacagaaa tgattatgta ctcttctcag 900  
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<213> Saccharomyces cerevisiae

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<210> 46

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<400> 46

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<211> 1110

<212> DNA

<213> Saccharomyces cerevisiae

<400> 47

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<213> Saccharomyces cerevisiae

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<210> 50  
<211> 942  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 50

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acctatcatt cagatgaatc tttaggcata aaacattcag attatataac ttcccaagat 180  
gaaagaaaat tgaggtcgga gatcatctcc gcagtgaaaa gagatctatc taaaaacaag 240  
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gtgaaaaatt tgtccaccac attttgtgta attcaaacac tgtgtccacc agagactata 360  
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<210> 51  
<211> 765  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 51

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gttttgata tcggtatggc tttagcttcc gccactgacg actcctacac tactttgtac 240  
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tcttctgctg ctccaacttc ttctgctgcc ccaagctcat ctgctgcccc aacttcttct 420  
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gctgctccaa gctccactgg tgccaagacc tctgccatct ctcaaattac cgatgggtcaa 660  
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<210> 52  
<211> 1407  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 52



<400> 53

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gactcattaa aacatatagt tgacgccaga aacagcttat cagagacact gctaaatagc 180  
aacgatgatg ggagtataca caattctgac cagaatactg gtttgaataa agacaaagag 240  
gcttcaatag cagataacaa tagtgctaac aagtgcgcca caagctcttc ccgttaccaa 300  
gagctcaaac aatttcttcc catttcctta gaccaacaga ttcatacagt atctttacaa 360  
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gaggatttca agattgagtt acacttggat ttgaacacaa aagaatatgt cgaagaccga 660  
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 tga 1863

<210> 54  
 <211> 474  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 54

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 ctggaagccc ttcttccctt acaaaagcct cttgggaatg aaggaggtaa cagggtacag 420  
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<210> 55  
 <211> 897  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 55

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aataacgtct gttcaactgt gactaagcca gtctcctcca aagctcaatc tacagctact 780  
tctgtcacat cgtcagcatc tcgtgttatt gacgttacca ctaacggtgc taacaagttc 840  
aataacggtg ttttcggtgc cgctgctatt gctgggtgccg ccgctctatt gttatag 897

<210> 56  
<211> 2508  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 56  
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ttgttccgtg gtgacaccgt tctcgttaag ggcaagaaga gaaaagatac tgtcttaatt 240  
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aagcaaatgg ctcaaattag ggaaatgggt gaactaccat tgagacatcc tcagttgttc 720  
aaggctatcg gtatcaagcc accaagaggt gttttgatgt atgggtcccc tgggtactgg 780





ttaggtacta cggctacgga caatgctaac agtaataata gtgctccaag tggagcaggt 2460  
gctgcatttg gttctaattgc ggaggaagat gatgatttgt atagtttag 2508

<210> 57  
<211> 651  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 57

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agctactcgc ttttgccggg cctcagcgca acgtatacgt cgatgataca acaagacgca 600  
tccaactgta cactgattac aacaagaacg gttcatcgga gcctcgacta a 651

<210> 58  
<211> 345  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 58

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ccttgcaaaa attgctatgt ttttcttgtg cttgggtattg cttcgtggag atatatattc 180  
tcatatcagg acggaatttt acaaagtgag aactctaaat ggtgtagtaa agagaagaaa 240  
aaaaaatgct cagcgattta cctcattac aaccaccgag attcactagg aaacggagct 300  
gtccctcgga atttattgtc aacatatcat ccaatgttaa ttagtag 345

<210> 59  
 <211> 552  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 59

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 tttttgttca tcatatcggt acatatctgt gaaaagtact ttatctcaat gggtttacgt 180  
 gggcatagat cacgcttcag ccgctctgtg tcgactttct tttcgccagg taaacttgct 240  
 tgcatagctc atctacgtgt aggctgccaa attgtaccca tatttcctta tgggtgctttt 300  
 ctgaagactc cttacaatag gtgcgcggga aacaaagtca gtgaaagtac gcatcgtaga 360  
 gctgtcgttc ggccatagcac tcgctatttc gtgacaacgt tccaggacac ggaaactcaa 420  
 ctcatatag tatcctctgt tgaggtaaaa aagagaaagg gtatcgtaat cttttctatt 480  
 gaatttcaaa gtatgcactt gaaacaacgt gtagaccatc aagttgattt tcttgggaac 540  
 aagatacttt ga 552

<210> 60  
 <211> 1599  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 60

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 tttggtttgg acctcgacct ggatcatctc ttgaaggagt tggactccaa tgtattggac 180  
 gcttggggcc aaatagagca tttgtaccca aaccagggtta tgagccttga aacttccact 240  
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<210> 65  
 <211> 1665  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 65

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 aagcgacaat gtgttgagga aaattacgat aaaaaatttt ctatgattaa aaaaaaacgt 180  
 cagcaaacat taaaaaagta caagcttagt cttctgaatc cgttggaag ggcttttcgc 240  
 cctttatcat atgaaaagta catgattggt ttaaatatgc agtatgcac gcattctctc 300  
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 agtaaattag tatttgcaac aaacaagggc tatgtcgcag gattttcatc cctggataat 480  
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 tataagtccg agccaactga tgtgttcaaa acaatgaaac ttgagagaa agttgccatc 600  
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<210> 67  
 <211> 336  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 67

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cccgttatg attctcgatg ttattatatt ctttag 336
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<210> 68  
 <211> 366  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 68

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gaagttagtt cggtacaaca tgtagtagat tctttttcat tctcgaaggc tggagaagta 180
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atttttgatt ttgaggttgt atatctgtat ttgtgtacta atgagtcaga tagcgataat 300
attatacgtc aaaaaaactt gggaaggaca atgaattgta ataacgctga tgatatgttg 360
ttttga 366
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<210> 69  
 <211> 597  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 69

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<400> 71

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<213> Saccharomyces cerevisiae

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<211> 462

<212> DNA

<213> Saccharomyces cerevisiae

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<212> DNA

<213> Saccharomyces cerevisiae

<400> 95

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 99

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 gaagatgtga ctggccaaca aatcgatttg aacaacagtg aaggtaatga gaattcagtc 1860  
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549

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<400> 102

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 <211> 810  
 <212> DNA  
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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 105

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<400> 106

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cgagagaaga aaaagacgtc taaaatgaaa atgataaaga cttcggatgg ccaaacaagg 1020  
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 caatcgttgt tggagccaga caaaaccgag gaggacgcaa tcaatcagtc aaaggaaaagc 1500  
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<210> 110  
 <211> 1770  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 ggtattgcct ccaagttgga gtacatcaaa gagcttggtc cccgatgccat ttggatctcg 180  
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 tggccaacct acggtacgaa cgaggactgc tttgccttga tcgaaaagac acataagctt 300  
 ggtatgaaat ttatcaccga cttagtcatc aaccattgct ccagcgaaca tgaatggttc 360  
 aaagagagca gacctcaaa aaccaatcca aaacgtgact ggttcttctg gagacctcct 420  
 aagggttatg acgccgaagg caagccaatt cctccaaaca attggagggtc ttacttcgggt 480  
 ggttctgcat ggacgttcga tgaaaagaca caagagtttt acttgcggtt gttttgctcc 540  
 acccaacctg atctaaactg ggagaacgaa gactgcagaa aggcaatcta cgaaagtgcc 600  
 gttggatact ggtagacca tgggtgtagac ggctttagaa ttgatgtggg aagcttgtag 660  
 tccaaggttg ctggtctacc agacgctcct gtgattgacg aaaactcaaa gtggcaactc 720  
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 tttagaaagg cacacaagga tattactgtg tatggatatg attttgagtt tattgatttg 1560  
 gacaataaga aactgttcag cttcacaaag aaatacgaca acaaacatt gtttgctgct 1620  
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<210> 111  
 <211> 2115  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 111

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 tctctagata tcaaaaacac tgtcttagat agtgcggatc tcaatgacat tcaaaatcaa 180  
 gaaacttcac tgaatttggg gcttcctcca ctatctttcg actctccact gcccgtaacg 240  
 gaaacgatac catccactac cgataacagc ttgcatttga aagctgatag caacaaaaat 300  
 cgcgatgcaa gaactattga aaatgatagt gaaattaaga gtactaataa tgctagtggc 360

tctggggcaa atcaatacac aactcttact tcaccttato ctatgaacga cattttgtac 420  
aacatgaaca atccgttaca atcaccgtca ccttcatcgg tacctcaaaa tccgactata 480  
aatcctccca taaatacagc aagtaacgaa actaatttat cgcctcaaac ttcaaagtgt 540  
aatgaaactc ttatatctcc tcgagcccaa caacatacgt ccattaaaga taatcgtctg 600  
tccttaccta atgggtgctaa ttggaatctt ttcatgaca ctaacccaaa caatttgaac 660  
gaaaaactaa gaaatcaatt gaactcagat acaaattcat attctaactc catttctaata 720  
tcaaactcca attctacggg taattttaaatt tccagttatt ttaattcact gaacatagac 780  
tccatgctag atgattacgt ttctagtgat ctcttattga atgatgatga tgatgacact 840  
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gataatttgg gcacatccac ttctggcaag aacaaatctg cttgcccaag ttcttttgat 1200  
gccaatgcta tgacaaagat aaatccaagt cagcaattac agcaacagct aaaccgagtt 1260  
caacacaagc agctcacctc gtcacataat aacagtagca ctaacatgaa atccttcaac 1320  
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tacgacctgg ttaataagca ggatgaagac cccaagaacg atatgctgcc gaattcaaata 1440  
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gaactcgagg agaaaccgtt ccactgtcac atttgtccca agagctttaa gcgcagcgaa 1980

catttgaaaa ggcattgtgag atctgttcac tctaacgaac gaccatttgc ttgtcacata 2040  
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<210> 112  
 <211> 375  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 112

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 agaatcaaaa agatcagtag agaaacgcct gtccagataa gtttctgggt gtatggaacc 180  
 ttcctttctg gagcaatcac ttccggcagg aaagattcaa atggcttaaa caagtctaga 240  
 acacggttgg aggacatddd caaagtaaag cgtgaaccag tgtcagtttt tctacttgct 300  
 actatattta actatgtttt tttcttttgc tttcaccaac tgaaccttgt agtaaattgga 360  
 gtgaggttga attga 375

<210> 113  
 <211> 1098  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 113

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 agtggggttca gcaattctac gattttgcag gagactttga actccaagaa tgccgtcaaa 180  
 gaatgtagaa ggttctacgg gcaggtgcca ttctgtttg atatgtcgac gacgtctttt 240  
 gcatcgctat tgctcgttc cagcatcttg agagaattcc tctcactatg ggttattggt 300  
 acgatctttg gtttactact ttacttattc acggctagtc tcagctacgt gtttgtgttt 360  
 gacaagtcga ttttcaacca tctcgttac ttgaaaaacc aaatggcaat ggaaatcaag 420  
 ttggcagtcg gtgctatccc atggatgtcg atgttgaccg ttccatgggt tggttatggaa 480  
 ttgaacggcc attctaaact atacatgaag attgattatg aaaaccacgg tgtaaggaag 540



ctcattatcg agtacttcac tttcatcttt ttcactgatt gcggtgtgta tttagcgcac 600  
 agatggttgc attggccaag ggtctaccgt gctctgcaca agcctcatca caagtggctg 660  
 gtctgcacac ctttcgcac tcattctttc catcctgtag acgggttttt gcaatccatc 720  
 tcgtaccaca tctaccatt gattctgcca ttacacaagg tttcttattt gattctgttc 780  
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 gccgtcaacg gtactgcctg ccacacgggt caccatctat atttcaacta caactacgggt 900  
 caattcacca ctctgtggga cagactaggg ggttcttacc gtagaccaga tgactcattg 960  
 tttgatccta agttaagaga tgctaaggag acctgggacg ctcaagttaa ggaagttgaa 1020  
 catttcatca aggaggtcga aggtgatgat aatgatagaa tctatgaaaa cgacccaaat 1080  
 accaagaaga acaactga 1098

<210> 114  
 <211> 1659  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 114

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 gatgatgtgg tcagagtagt cagccatagc gatgagagta ctgatgacga actttgtaat 180  
 gtgaatttaa cagaaacagg ggcaatcttc acgagtaaag gttttaccgg gttaagcaaa 240  
 ggtttcacag ataagaccct ggatttcctg gtacgagtgg ccggttcgca ggcggttttt 300  
 tttattgttt ggatcatcct cataatttgg gtgggttattg gtattgttta taacgcacct 360  
 ttcaattggc aagttgttat gcaggacgga cagtctattc aaagttatgt ttgggacaca 420  
 ctggtgatga gacaacagtt gatgagtacg catgaacaaa ttttgatctg cggtagattg 480  
 aagtcgagat tggcttcctt caaaaactat ctaacaagaa gcacccaga ggaagaaaaa 540  
 gcagactgca cagttgaagc taatgaagtc agctctgttg aaaatcatat agacccatct 600  
 gccattaacg gagaactgcc tgtggaaaat tggtagacc gtttatctaa cgtagcaagt 660  
 aggtatatgg gttcaattgc agcaatggtg atattttgga taggtatttt cgtttggatt 720  
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ggtagtaatc caagattgaa aaagttcagt gacgcttggc agatgtatat taacactgca 840  
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tgtattgctt cgggtttgct ttggagtaca acaggccaat tgattgctaa cagccaact 1500  
atgattatcg aagaattttt ctgctagtt ttgttgcaag cacataattg ggccgatcgt 1560  
caaagaagag tggagggttac cgctttgtac gcacgtaggc gcatacttct atcatacgta 1620  
gaaaagcggt tcccagaggt tatgatgttg gaaaaatag 1659

<210> 115  
<211> 1722  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 115

atggaaacta ttttgcagcc aaaggctaga ccatttgagt ctttgaaaag aaaacgtttt 60  
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ttgcgtgact ttgcaaaacc taatcccgt gacacatttt ctaatcttga ttctgggtcat 180  
tgtccttttg tcacaactcc aataaaatat gagtgccag atggaaagag ttcttttttc 240  
cgaggagaca cttaaatttga aaccctgttc agtaatagaa aattctatga gttcaaagat 300  
aatttgaaaa ggggattgaa gaaaatacgt catgggagaa acggacatca aagcgaaaag 360  
agatgtccag ttgttgaaga aacaaaaaag tctgtgtcag ataactctgga caaaccagac 420  
aataatacgc cctgttttga cagattccac acaaatcga aagaatttga aacgcaattt 480

gatcattcaa ataggagcca aaattccgag aaggcttatc tagacaatga atcctggttg 540  
aacctaagtg agaaatttat tccttttaaat aattttaaata atgaagattt gaaacatttt 600  
gaagagaatt tgcaaagctt agcgcttgca acttttactc caattgaatc aaatgaatcg 660  
cttgataggt cagattcgac acgtggcaca aaacgaagca ttcgcaatga ttccagtgat 720  
acaacatctg aaaagagggt atgcttaaaa caatactcag atgaacctga atcggatcat 780  
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ttaagctcca cggattcggt tttaattgaa aaagtagatt ttccctctaa caaaattggt 900  
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actgaaatga ggtcaattct aaagacaaag atgaattcac agcatgatga ggagtctcag 1620  
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gagtataaaa ggcagagaaa tgaagcagaa attatagact ga 1722

<210> 116  
<211> 618  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 116

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gcactacca catgtggctc aggggttattt atgctcactt caagcgtgcc attgttacag 120

gaagtgtgcg acgcgtcggg tacgctagca tgcaccgctt cattgttcac aagcagcggc 180  
ggctttttta gcaaaccacc tgtagttcca ctagattttc tacttctgct tctacttcta 240  
ctattaccgt tgctattgcc accgttgccg tccgttaaag gtgagccaga cgcatgcgag 300  
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aactttgctt cccactcttt cactactgac catcgatcct tcttatttca tagcctcacg 480  
agcacaaatg ataatacgag aaaaaggccc gaccgggtaa ccaacccttt tactatctct 540  
cgctctacct ttagtaataa tgcgtctat ataaggattt actcatacag tagtccaaaa 600  
tatacctttc cgtgctaa 618

<210> 117  
<211> 534  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 117  
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tcgttactaa gagtagatat agtcatttct atttgttacc acacacaaat atatcttcat 180  
ccggctgata tttgtctcta ctgccccctt gcttgcaatt taatgaccaa attacacatg 240  
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aagggccgta cacacaaacc caagccgagc gaaaaacaca aaaaaataa aacagggaaa 480  
aaaggagcgc aagagaaaac gcacaggagt aggagcagca gaaaggggaa ctaa 534

<210> 118  
<211> 1833  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 118  
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gaaggagtggt agtcgactga ggcggaacgt gtggcagggga agcaggagca ggaggaggag 240  
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aggttccacc gaaaggatgc tagcaagtat gtgtcgtttt ttggggacgt gagttttgat 360  
cctcgcccca cgctcctgga cagcgccatc aacgtgccct tccagacgac tttcaaaggt 420  
ccggtgctgg agaaacagct caaaaattta cagttgacaa agaccaagac caaggccacg 480  
gtgaagacta cgggtgaagac tacggagaaa acggacaagg cagatgcccc cccaggagaa 540  
aaactggagt cgaacttttc agggatctac gtgttcgcat ggatgttctt gggctggata 600  
gccatcaggt gctgcacaga ttactatgct tcgtacggca gtgcatggaa taagctggaa 660  
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aagtggactg ggttcgttgc agtgagcatc ttcgagttgg ctttcatccc cgtgacgttc 840  
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tatctgttca tgttccaact gtcgcagttt gtgtggactg ctttgagcaa caccaagttt 1740  
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agtatcatta tgacgttgta cctgacctta tga 1833

<210> 119  
<211> 3363  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 119

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caggcgaccc ctgaatgcc aagagtttcc tctaagtatg atcctgataa cccaaacaaa 180  
gataagttgg gaacatacga tggggatatt gtgcctactg ctttaaactg attgtctatc 240  
cttatgtttc ttcgttttgg cttcattttg ggtcagttag gtattatatg caccatcggg 300  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*



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<211> 381  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 122

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<210> 123  
 <211> 1527  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 123

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 <211> 2586  
 <212> DNA  
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 <400> 124

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 <213> *Saccharomyces cerevisiae*

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 caatatacaa ttgtttcttg a 321

<210> 126  
 <211> 1482  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 126

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 <213> *Saccharomyces cerevisiae*

<400> 127

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 ccatctactg actacaccac tgactacact gtagtcactg aatatactac ttactgtcca 540

gaaccaacca ctttcaccac aaacggtaag acttacaccg tcaactgaacc aaccacattg 600  
 actatcactg actgtccatg caccattgaa aagccaacaa ccacatcaac caccgaatac 660  
 actgtagtca ctgagtacac tacttactgt ccagaaccaa ccactttcac cacaaacggt 720  
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 gtcgtcccag tttcatcctc tgcttcttct cattccgttg tcatcaacag taacggtgct 960  
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<210> 128  
 <211> 1386  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 128

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 aaagcgggtt ttcaaagtta cgggtgttagc aaggaggctg atatgggcaa tgataaaata 180  
 cacaaaaata gcgtgaaatt gcaaaagaag ctcgctaaac tgttcaagga atcgaaaaac 240  
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 ctagtaaaaa aggtttttct acagatctgt tctgctctta attattgcca tgagcacggc 540  
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ctttccgatg acttttactc attattatct aagattttac aagtgaatcc gaagaataga 960  
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 gacgatgata atggcagtcg cagtggcagt ttcggaacgc tagacacaga cactggattg 1260  
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 aactaa 1386

<210> 129  
 <211> 2280  
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 <400> 129

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 caagagccta gcgctaccaa gaagatactt tactccatcg ccacatggct gttgtacaac 180  
 atcttccact gcttcttttag agaaatcaga ggccggggca gtttcaaggc accgcaacag 240  
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 ttaaagcaac ccccatagg gtttttggtc agtttcttca tggccatagg cgtggtaagg 420  
 ccgcaggata atttgaaacc ggcagaaggc actatccgag tagatccaac agactacaag 480  
 agagttatcg gccacgacac gcatttcttg actgattgta tgccaaaggc tctcatcggg 540  
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<400> 130

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 tgtgaagttg accctacatc tggcgatttg gttgaaaaac caaagaatca taagcatcac 1800  
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 <211> 1089  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 131

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 tcggaatact gtatatccag tgacgcagga acagagaaga tggatagcga cgaggagaag 180  
 tcgttggcca atctgccgga gttgaaatac gctcccaagc tatccagcct ggtgaagcaa 240  
 gagacgctca ccgagagctt gaaaagacca cacgaagatg agaaagaggc gatagatgag 300  
 gccagaaga tgaaagtgcc gggagagaac gaggacgaaa gcaaggaaga ggaaaagagt 360  
 caagaactgg aagaggcaat tgacagcaag gagaagagca ccgacgccag ggacgagcaa 420  
 ggggacgaag gtgataatga ggaggaaaac aacgaggagg ataataaaaa cgaaaacgag 480  
 catacagcac cgcttgcgct ggtgatgccc tcccccatcg aaatggagga acagaggatg 540  
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 ccggaattgc aggtctacta ctcgaagatt gccgcatcc gtgactacaa gctacaccga 720  
 gcgtaccagc gacagaagta cgagctttca tgcatacaac cagaaacaat cgctaccagg 780  
 acattcattc accaggactt ccacaagaag gtcaccgacc tgcgagccag gctgctgaac 840  
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 ccagatgtca attaccacgt ccccatcaaa cttgataaca agacgctgag ctgtatcacg 960  
 ggctacgcag cgcacgacag ctgtgctatc ccggcgagcc cgtggcagag gacctcgctt 1020

gcgaaagcat cgagtaccgc tacagagcca acccggtgga caaactcgaa gtcattgtgg 1080  
accgaatga 1089

<210> 132  
<211> 984  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 132

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aaagatagac tgacagcatt gcaaacggat ttaacttctc tgcatacaagg tgataatggc 180  
caatatgccc gccaaagtacg agatttggag gaagaaagag atctagagtt agtcagggtg 240  
cgctgttttg aagagtaccg tgtttctcgt tccggtatcg aatttcaaga agatattgaa 300  
aaggctaagg ctgaacacga gaaactcatt aaattatgca aagaaagact gtattcgtct 360  
atagagcaaa aaataaagaa attacaggag gagagggttac taatggatgt ggccaatgtg 420  
cactcctatg ccatgaatta tagcagaccg caataccaga agaacacgag aagtcacaca 480  
gtaagtgggtt gggattcttc gtccaacgaa tatggaagag atacagcaaa cgaaagtgct 540  
actgacacgg gagctggaaa cgatagaaga acgctaagga gaaggaatgc ttctaaagac 600  
acgagaggta acaacaacaa tcaagatgaa tctgattttc aaactggtaa tggttctgga 660  
agtaatggcc atggttctag acaagggtcg caattcccc atttcaacaa cctaacctac 720  
aagtcaggca tgaactctga ctacagattt ctacaaggta taaatgaagg tactgatcta 780  
tatgcatttt tgtttggcga aaagaacccc aaagacaatg ctaatgggaa tgaaaagaaa 840  
aaaaaccgtg gtgctcaacg atattccacc aaaacagctc cacctttaca gtctttgaaa 900  
ccagatgagg tcacagagga tatttccttg atcagagaat taacagggtca gcctccggct 960  
cctttcagac taaggtctga ctaa 984

<210> 133  
<211> 996  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 133

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attcgtcagg gatccttgaa tgggtcccaga ttaattacgt gtggacatgc catttcccaa 180  
actggtgggtc atggcgatct gagatctggt gccctacctg ctagtgcctt tgacagctgt 240  
tcatgccact ttgggtcaagt tgggtgttgta gcagatgggtg ttcccgaatg ctacaaagcg 300  
gccagagaag agtttagaag aggtgcagac tttattaaga ttatgggtgg tggaggtgtg 360  
gcctctccaa ctgacaaaat atcaaacaaa caatttttgcg acgacgaaat aaaagcactt 420  
gtagatgtcg caaatagtta ccacacatac gtaacagcac acgcctacac tgccgaagcg 480  
atacaaaatt gtatcaagtt aggtgttaag ggtatcgaac acggaaactt attagatgaa 540  
cgtaccgcag agcttatggc agaattgggt tgctacttga ctccaacact agttacctac 600  
aaagtgatgg gttctgatca atttagtgca ttcttgggac ctgaaaatag tagaaaaaat 660  
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attagaggga aagttcaaac gacacaagag attttgctct cagcaactgt tactcctgcc 840  
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atactatttg tcatgaaaga gggaaggata tattga 996

<210> 134  
<211> 1215  
<212> DNA  
<213> Saccharomyces cerevisiae  
<400> 134

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tttgagagcc agatgtcgtg gctaagggtt caaacaaggc agtatctaac tagattcaca 180  
gacaaccaat cagatttcgt acattcttta caaaaaagc acagaacgcc ttttagagac 240  
gtttatttca aatacacttc gcttatgggt tcccacatgt tttatgttat cgtgcttccc 300  
atgcctgtgt ggcttggata ccgcgattta acacgggaca tgatctacgt tcttgggtat 360

tcaatttatt tgagtggcta cttaaaggat tattggtgcc taccaaggcc aaaatcaccg 420  
 ccagttgaca gaatcacact aagtgaatac actacgaaag aatatggtgc acccagttca 480  
 cattctgcta acgctactgc ggtaagtcta ttattctttt ggagaatatg tttatctgac 540  
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 gaagaagaag agtgtttatt gtacagcggg gtttccaaag tggaaatcgt cggaagggtt 1140  
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<210> 135  
 <211> 429  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 135

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 gcaacttcca actcttttga cgtaaacaca ggtgctgatt ctttcgagga aggggaggag 300  
 gaggaggagg aagaagacgt atatctcttt ctgctgcttc caatgattcc agcaacggta 360  
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 attagttga 429

<210> 136  
 <211> 1548  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 136

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 gaagaacttt taaatcaatt gaacgggtact tcagacgatc cagtgccata taccttcagc 240  
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 agagggcata tagccagcgt ataccagggt gcgtgggtcat cggactgccg actactgggtg 1380



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gtgtgtagtg gtgggaaaga caagatggta agattgtgga cgcattga 1548

<210> 137  
<211> 1731  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 137

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ctaagacaag tacgtaccgc taataagatc ggtgctgtct ttgttcccgt cggcgggtggt 720  
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 cttactttat tccattatag aaaccatggt gccgatatcg gtaagggtttt agctgggtatt 1620  
 tccgttcctc caagggaataa cttaaccttc caaaaattct tggaagattt aggctacact 1680  
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<210> 138  
 <211> 3570  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 138

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 gcgcaaagcg ccggagatgc ctctttgcag tatgctaata tgagatctgc caatgtgagt 180  
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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 140

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 <211> 612  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 142

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<210> 143  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 143

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 <213> *Saccharomyces cerevisiae*  
 <400> 144

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tcaccggcca caatgcagac gatatggcag agacagtact aa 582

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 <211> 1422  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 145

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<210> 146  
 <211> 4104  
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 <213> *Saccharomyces cerevisiae*  
 <400> 146

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 <211> 351  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 149  
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 caagctaact ctttagatac ggattacggt ttaacaaggc ctagcaatgg gcgcgttcca 540  
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<210> 151  
 <211> 3042  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 151

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<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 152

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<210> 155  
 <211> 1644  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 155

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 aataacaacc cgcccggaaa ttaa 1644

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 <211> 1761  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 156

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 <400> 159

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 <400> 161

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 cagtacataa aagcacgtat gaaagataag accttcttct atacgaagca attccgtaca 180  
 gccaaaaaca aatttttctt tcattctgtac cattgggagg ccactcatat taacgttgac 240

cactatatat gtacatgtca tcccattttt tggggctcta taggtcagaa actcaggaga 300  
tccgcctga 309

<210> 162  
<211> 2835  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 162

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<210> 163  
 <211> 1023  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 163

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gaatacatcg acttcaagcc tcgattgaac aacgatctat cttacaagca ataccaaaga 780
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tacttgcctt ggccagaacc agaagagggt tctgatcctt tggaagctca aagattgatg 960
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<210> 164  
 <211> 2781  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 164

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 ggtaaggcaa aagatcaaga tttgagcagt cttctagata aatgcatcga tattttatct 180  
 atttacaaga agaactcgaa agatatcaaa aatattatat cgtgcaaaaa taagggtgca 240  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 165

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<210> 166  
 <211> 534  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 166

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<210> 167  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 167

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<210> 168  
<211> 363  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 168  
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<210> 169  
<211> 1845  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 169  
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attctgccta tggatggaca gtacagaaga acatacattt ctgagaatgc attgatgcct 180  
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<210> 170  
 <211> 510  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 170

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gtcatcggta tcagtgcata cttcttgatg gtaccctccg tagttcaaac gaagaaacct 360
tggagcaaga aaggatgggt cactgagagg gaagaaaaaa tcatcgtaaa caagattctg 420
agagatgatc cgacaaaagg ggatatgaac aataggcaag gtatgtcact taaaatgtta 480
tggcagggga taacagatta ctatatatag 510
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<210> 171  
 <211> 609  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 171

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acggaagtgc ggtcattcta cgaagacgaa aagtctggcc taatcaaagt ggtaaaattc 180
agaactgggtg caatggatag gaaaaggctt tttgaaaaag ttgtcatttc cgtcatggtc 240
gggaaaaatg taaaaaagtt cctgacgttt gttgaagacg aaccagattt ccagggcgga 300
ccaatccctt caaagtatct tgttcccaag aaaatcaact tgatgggtcta cacgttgttt 360
caagtgcata ctttgaaatt caatagaaag gattacgata ccctttctct tttttacctc 420
aacagaggat actataatga gttgagtttc cgtgtcctgg aacgttgtca cgaaatagcg 480
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609

<210> 172  
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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 172

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aaactagcat gccagaattg ccgtagaaga agaaggaaat gtaacatgga aaagccttgt 180  
tcaaactgta tcaagtttcg taccgaatgt gtattcactc aacaagactt aaggaacaaa 240  
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caatcagcac taccatcatc tgaaagcaac gatgagaatg agtctgatgc gttcaccaag 480  
aaaatgcctt ccgagagccc tccaccagtt ggcacaaata gtatatatcc atcaaattcc 540  
ttgtctataa taaaaaagaa gacggatgga agcacaagat atcagcaaca gcaggtcagc 600  
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ctatacccg gacattacct tttcattcat agagaaactt tcttaagtgc tttcttcggt 720  
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 aacaccaatg atggtttgtc gaagtaa 1947

<210> 173  
 <211> 1461  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 173

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 atgtacacta gtgggaagga gatcaggaat aagaaaggta atttaattag ggccgcttct 180  
 ttccaggact ccacaatacc ggatgagagg gtccaaccag atcgtcgttg gttcggtaac 240  
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 aaggacgcag atgaatcccc aaaagccaga attttgata ccgaaagtta tgctgatgcg 420  
 tttgggcca aagcccaaag aaagagacca cgtcttgctg catccaatct agaggacttg 480  
 gtcaaggcta caaatgaaga cattaccaag tatgaggaaa agcaagtctt agatgccaca 540  
 ttaggactaa tggggaacca ggaagacaaa gaaaatgggt ggacctccgc agcaaaagaa 600  
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gacattttat tcagaggtgt tgtcagagtc gaacacgtca cacaccaga acaatatatt 1200  
ccaggtgttt taaaacgttg tcaggtaaag cacttggaag gaacttacga gatctcagga 1260  
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ggtggtgagc ctgatgagtc tgggtgttct aagcaaata tgaatgattt taatagaggt 1380  
aaaatccctt ggttcgtcct tccacctgaa aaggaagggg aagaaaaacc aaagaagaaa 1440  
gaagttgaga agacggcata g 1461

<210> 174  
<211> 1074  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 174

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ggtatctgcg gctctgatat tcattattat agaagcgggtg gtattggtaa gtacatattg 180  
aaggcgccaa tggtttttagg tcatgaatca agcggacagg ttgtggaagt tggatgatgcc 240  
gtcacaaggg tcaaagttgg tgaccgtgtt gctattgaac ctggtgttcc tagccgttac 300  
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cactctaata aattggctgg ggtccgcttt ggtaccaaag ttgtgtatt tggatgcaggt 540  
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gtcaagccat tgataacca caaatTTaaa tttgaagatg cagccaaggc ttacgactac 1020  
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<211> 3306  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 175

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ttttcgtcaa cattcagtga tgatgatcgt tcggaccttg ttgctgtacc taatgagtcg 180  
ccgcatgcat tttcgtataa tcccatatca ccaaactcac tgggagtaag gttgaccatc 240  
ttaagaaggt ctttggaat aatggtaaac agtcctgaca tcttacatga gttgaagaaa 300  
aaagcaccgc taatagcata cccccctca cttagacaca caagaaactt aacagagact 360  
gccacgttat cagcatcgcg agatccgtta aatgggtctc taatttcacc attagtatcc 420  
aatatgccat ctctgctag tagaccgtg atacagagag caacgtcctt aatggtgttg 480  
cctgataatg atactgccag taaactgaat ccagccaagt ccgagttaga aaacttgta 540  
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 aaccttttga gtcgcacaga aaataatagc aatcataatt acaacaacag taatgtgagc 1320  
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 agaataacaa attctatgaa gattgacctg ttaaaggcgg attacacaag aagtctatta 2160

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 <211> 393  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 177

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 acgatagctg ctgtcttttc tgtacgtgta taa 393

<210> 178  
 <211> 2304  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 178

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 aatgacttct ctttttacga ccaagttttg gatttgtctt tgttggtcaa tgtcattcca 240  
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 <211> 816  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 179

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 acggaagtgc ggtcattcta cgaagacgaa aagtctggcc taatcaaagt ggtaaaattc 180  
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 gggaaaaatg tacaaaagtt cctgacgttt gttgaagacg aaccagattt ccagggcgga 300  
 ccaatccctt caaagtatct tattccaag aaaatcaact tgatgggtcta cacgttggtt 360  
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 ttacctggcg aaaagaaagt cgacacagag cggctgaagc gtgatctatg ccacgtaaa 720  
 ccactgaga taaagtactt ttcacagata tgtaacgata tgatgaacaa aaaggaccga 780  
 ttgggtgatg ttttgcattg gtgctgcca agttga 816

<210> 180  
 <211> 1965  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 180

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attacagtcc ctagatcacc aacttctctt tcaaggaatg ctcaaccaac tacttttaaaa 180  
cggcctccat tatcttcaag accatattca tataataccc caaccaaaga taggaaatcc 240  
ttttccaaat ccgcgaaaca gaacaataac aataacaacg ctaattccgg aacatcgctt 300  
cacgcagagt tcaaaaatta cagagatatg tttttatcta atagaaatgg tttcacaggt 360  
agggttttcg gtgttacttt agcagaatcg ttgagcgctg ccagtgcaga ggtcattgtt 420  
caaagtgagt tggtagagtt tggtcggata cccatcgctg tagccaagtg cggcgcatac 480  
ttaaaagcaa atggattgga gacctcgggt atatttcgta tagcgggcaa tggtaaaaga 540  
gtaaaagccc ttcaatacat attctcgtcg ccacctgatt atggtaccaa attcaacgat 600  
tgggaaacat atacagtgca cgatgttgca tcgctcctga ggagatacct taataatttg 660  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 181

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<210> 182  
 <211> 1563  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 182



<210> 183  
 <211> 1770  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 183

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<211> 2196  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 184

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 <211> 2790  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 185

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 <213> *Saccharomyces cerevisiae*  
 <400> 186

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<213> *Saccharomyces cerevisiae*

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<210> 188  
 <211> 648  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 188

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 gcagatgtgg ttgtgtataa cagctatctg aaaaaactcg aagaagcaaa agtaaaagaa 600  
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<210> 189  
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 <213> *Saccharomyces cerevisiae*

<400> 189

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<213> *Saccharomyces cerevisiae*  
<400> 190

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 <213> *Saccharomyces cerevisiae*



<400> 192

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<210> 193

<211> 3303

<212> DNA

<213> *Saccharomyces cerevisiae*

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195

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 195

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 <213> *Saccharomyces cerevisiae*

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<211> 2922  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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 <213> Saccharomyces cerevisiae

<400> 203

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 atcatcagag ctggtaatta cgctcttcat ccagaagtgg ttagagaaca agtaaaggat 1020  
 cccagaacct tgataggcta tggtagattc ttcatctcta acccagattt agtctaccgt 1080  
 ttagaagagg gcctgccatt gaacaagtat gacagaagta cttctacac catgtccgcg 1140



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<210> 215  
<211> 354  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 215

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tatgtatacg aacagtatga tactgaagat gacaaggtaa tgcattcattc tatacgtgtc 180  
attctgaacg aggcgcgctt tccttttttc tttttgcttt ttcttttttt ttctcttgaa 240  
ctcgagaaaa aaaatataaa agagatggag gaacgggaaa aagttagttg tggatgatagg 300  
tggcaagtgg tattccgtaa gaacaacaag aaaagcattt catattatgg ctga 354

<210> 216  
<211> 1575  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 216

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agagatgaga aagggtgattg caatgaagaa aaggattctt ccaaagattt ggggagggtta 180  
ccgtcgaaga tgaaacgagc atatgatggg gaaacagtta ttaaagaggg agattcgcac 240  
gctgagtcgc tagcgcagca gggaaagcag cccacagacc tcgcatataa cagcagatcg 300  
aagatatctg gttctaattt gcatttattg gttcctagag ttgcgtctac agactatatt 360  
tcgaataaag aggttcacac ggagggcctg tttgccggct atcgaccctt gtttctgggg 420  
aactcagggtt ttccgtctga tgcaagaaag ggtaaaaact ttcattgagtt agacgacgtt 480  
cttccaata tacaggtagt ggacgcttcc gagaaagatg gcaaactcaa tgtgcaggag 540  
attattgagg acttacaag aacaagtttg agagaaagca ttcattagtat ggaacagtta 600  
ccatcttcgc acaaacgtaa acccgtaata ccgtgggacg catctataag tggcatgggt 660

tataatgaca tgcctttcaa atatgtgccc aaaaatatta ttctgaaaat gaagccattt 720  
 aaacttttgc gtattgagag aaaaagtcaa gcgaagaatg caagaaagcc tactatgata 780  
 aaacttcagt ttcacaatcg aagaatcaat gacaccccag agttagtga tttataccat 840  
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 tcaagtgcaa atacaagtaa aagacagaaa atgttaaaag caagaagcga ctttgaacat 960  
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 aatgaactaa ctaagttaaa taagatactt gctagagaat tcaaaaaatt gacaaagcta 1080  
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 aacaaaatca aggcgtatat tgaaaaaatc atagtgcggt tatcagatga agtaccatcc 1320  
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 cataaggacg ttcaagtcag cttcaatgac aagtatgttg ttactaggag cggcgtgagg 1500  
 tatacgaggt atcccactaa tttgaatata caattattgg aaactgcatt tgaagaatgg 1560  
 gactactatg agtga 1575

<210> 217  
 <211> 1557  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 217

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 aatgctagta ttcccaaaac tccggaggac atctctatct tgcccgtcaa tgatgaacca 180  
 ggttaccttc aagattcgaa gactgaacaa aactatcctg agcttgccga tgctgtgaag 240  
 tcacaaacaa gtcaaacatg cagcgaagaa cataagtatg ttatcatgat cgatgccggc 300  
 tctaccgggt cccgagtaca tatatacaag tttgacgtct gtacttcccc acctacatta 360  
 cttgatgaaa aattcgacat gttagagcct ggtttatctt ctttcgatac cgattccggt 420

ggtgccgcta actcccttga cccattactg aaagtagcaa tgaactatgt ccctattaag 480  
 gcaagaagtt gtactcccggt tgcgggtgaaa gctactgcag gcctaagact cttgggtgat 540  
 gccaaatctt caaaaatttt gagcgctgta agggatcatt tggagaagga ctatcctttc 600  
 ccagttgtcg aaggtgatgg tgtttccatc atgggcgggtg atgaagaagg tgtcttcgcc 660  
 tggattacta caaactacct attaggtaat atcggtgcta atggcccaa gttacctact 720  
 gctgccgttt ttgacttagg cggagggtct acacaaattg tttttgagcc tactttccca 780  
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 aactctgttc ttgtggaaaa tgcattaaaa gacggcaaaa tattgaaggg tgataacact 960  
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 ggtgcacaat gtagatttct gactgatgaa attttgaata aagatgctca gtgccagtct 1140  
 ccaccatgct ctttcaatgg ggttcaccaa cttcttttag ttctgtacatt caaagaatcg 1200  
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 caaagagaat tgagaactgg aaagaaaatt gccataaag aaatcggttg gtgttttaggt 1500  
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<210> 218  
 <211> 552  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 218

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 ctggctggag cagcaactct gttagagaca ataacgttct tgatttcagt caatgggttcg 180  
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aagtcggata agaaacctct gatggctctt ctaaccatgg tgttcttacc catcaagacg 300  
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acaccaacaa cgaacaaaga cttgtattct tccaagtatt ctcttaattt agcaaagtat 420  
tcagctttct tttcacgaat gcctcccatt tcaaacttat tatacgtatt tattagactg 480  
tttgcaggat gtttaaaggt attccgctta tgtatcttat ggctgaaact tgaaaaaaga 540  
atcgagaatt ag 552

<210> 219  
<211> 663  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 219

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attgagacac aactagaggc gtatttcagt gtgcttgagc agcaaggcat cggcatggac 180  
tctgcgttgg tgacgccaga cgggtatcct cgttcggatg tcgatgtatt gcaagtcact 240  
atgatcagaa agaatgttaa tatgctgaag aatgatttaa atcacctttt gcaaagatca 300  
cacgtcttac taaaccagca ctttgataat atgaacgtta agtcaaacca agatgcaaga 360  
aggaataacg acgatcaagc tattcagtat accatccctt ttgcatttat cagtgaggta 420  
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ggtaatgtgc atgcggcaaa ccaactctaaa cttcaaaaca ttcagatggg tgtaatgaaa 540  
aatgaagaca ggccacttcc cgtccttctc ttgagagaag ggcaaactct aaagacatcg 600  
ctaacacctt cgagaaactg gaatggtaga ggtcttttgg gttgtaggat acaagagcta 660  
taa 663

<210> 220  
<211> 2295  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 220

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tccacccacg ctcccataga aaatgacacg tttttcgagg atgctgataa agtcagtttc 180  
atcaatcacc ccttagttct tgatgatata gaacagcata tcatcagatt gaaatcactg 240  
ggttacaata ccattcgttt acccttcacc tgggaatctc ttgaacatgc tgggtccagga 300  
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caacaaggta tgtacattta tttggaccct caccaagacg tctgggtctag gtttagcggg 420  
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tgcgcaagaa ttaaagaaaa ggctcctgag ttgtttgaga gcaactgcat tattggatta 780  
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 ccaagtttca taaaaccaga taatcattta gatttgata gtccctcgtg cactttaaag 1920  
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 cacggaagat ttgagtttgc tgagtttaac ttatgtaata aatcctacct tttgaaatta 2040  
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 gactaccagg ttcttgaatg gtttcacgag cctggccatc agttcattga aatttgcgca 2220  
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 tgcgttatca gctaa 2295

<210> 221  
 <211> 3123  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 221

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 gacggtggca atttcccaat gtatattgcc attaatgagt attttaagcg aatggaagat 180  
 gaactagata tgaagcccgg tgacaagatc aaagtaataa ctgatgacga agaatacaag 240  
 gatggctggt attttggtcg aaatctgaga accaatgagg aaggtttata tccggtggtc 300  
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 aacgattcaa acagtgaact gccaaccccg caaccaattg aaaccgccgc ctctatttcg 480  
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 taa 3123

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 <211> 2682  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 222

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 gaaaataatg acacgtttta tgataaggac ttagagagca attctgtagc aaagcacaac 180  
 gcagtgaaca gctcaaaggg cgtaaaaggc tcgaagattg actactttaa tccttcagat 240  
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 cttattgcag gaattacat tggctgtgtc ctctgcccgc aatccatgtc atatgcacaa 480  
 gcgcgccgcg taccagcgca gtatgggttg tactcctcgt ttattgggtgc ttactcctac 540





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<210> 223  
<211> 1908  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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<210> 224  
<211> 3189  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 224

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agaatattgt ttgtctgcca ggcttgttgg aagtcaaaaa caaagtgtga tagagaaaaa 240  
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ccagcaccac gaattccgag taaagacgcc attatatcaa gggttgaaaa agatatgttt 360  
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 agatcagcaa gtccgatcaa tacaaacaat gctagcgggg acagtcctga taccaagaag 480  
 cagcataaaa tggaacctat atatgaacaa agtggtaacg gggatataaa caatggtacc 540  
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 cgcccaaac gctactcgtt gctgttacta ataccaaacg catccgaatt gtcagaaaca 720  
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 aactactctc gctattatth ccaccattht ttgcagttgg ggttcagtga catcctagtt 1020  
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 <211> 1698  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 226

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 actaggacag gcgcaaatca attcaataat aagtctcggg ctaaaccaat ggagattgtg 180  
 gaaaaactgc aaaagaagca aaaaacgtcg tttgagaatg ttagcactgt catgcactgg 240  
 tttcgaaatg atctacgttt atatgataat gtgggactgt acaaaagtgt tgcgctcttc 300  
 cagcaattga ggcaaaaaaa cgcaaaagcc aaattatatg ctgtttatgt catcaatgaa 360

gatgattgga gagcccatat ggatagcgga tggaaattga tgtttataat gggggcggtta 420  
aaaaatttgc agcagtcctt agccgaatta cacatacctc ttcttctgtg ggaattccac 480  
actccaaaaa gtaccttata taattcaaaa gagttcgtgg agtttttcaa agaaaaatgt 540  
atgaatgtaa gttcaggaac aggtacgata atcactgcta atatagaata ccaaacagat 600  
gaactgtacc gtgatattag gctggttagaa aatgaagacc atagattgca attgaaatac 660  
taccacgact cttgcattgt tgctcctgga ttgatcacta ctgacagagg caccaactat 720  
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<210> 227  
<211> 2772  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 227

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<210> 228  
 <211> 3246  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 228

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gaaaaaagaa ccgctaagcc tatgattcaa aaggccttga cgaatacggg taatttcatt 240  
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<210> 229  
 <211> 4146  
 <212> DNA

<213> Saccharomyces cerevisiae

<400> 229

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<213> *Saccharomyces cerevisiae*

<400> 231

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 <213> *Saccharomyces cerevisiae*  
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 gaacgtgcag tagatactgc tgaagaattc gaagatgatg attgcacgaa aaattgtgcg 300  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 233

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<213> Saccharomyces cerevisiae

<400> 234

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<211> 1692  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 238

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<210> 240  
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 <212> DNA

<213> Saccharomyces cerevisiae

<400> 240

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<210> 241

<211> 4263

<212> DNA

<213> Saccharomyces cerevisiae

<400> 241

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 tcaagggatt tatggtgttt cagtataaat gatgatccgg taccgacacc tctgcgata 240  
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<212> DNA  
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<211> 1425  
<212> DNA  
<213> Saccharomyces cerevisiae

<400> 243

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<211> 243  
<212> DNA

<213> Saccharomyces cerevisiae

<400> 244

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<210> 245

<211> 483

<212> DNA

<213> Saccharomyces cerevisiae

<400> 245

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<210> 246

<211> 2424

<212> DNA

<213> Saccharomyces cerevisiae

<400> 246

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<211> 1668  
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<400> 247

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<211> 1956  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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gaatttacag atccagaaac caaactaaca catttctata atgatttgta ttcatactcc 360  
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<211> 2088  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 249

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<210> 250  
 <211> 2709  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 250

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<213> *Saccharomyces cerevisiae*

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<210> 252  
<211> 765  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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 <211> 363  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 253  
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<210> 254  
 <211> 1845  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 <211> 2580  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 255

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 <211> 1710  
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 <213> *Saccharomyces cerevisiae*

<400> 256

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<211> 3369  
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 <213> *Saccharomyces cerevisiae*  
 <400> 257

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<400> 259

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 <400> 262





<210> 263  
 <211> 2169  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 263

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<213> *Saccharomyces cerevisiae*

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<213> Saccharomyces cerevisiae

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 <211> 3396  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 280

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<210> 281  
 <211> 1674  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 281



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<210> 282  
 <211> 1185  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 282

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<210> 283  
 <211> 987  
 <212> DNA

<213> Saccharomyces cerevisiae

<400> 283

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gctggctatag tttcataccc agaaaagaga aattcatcaa ctgcaaataa agaagatggg 180  
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<210> 284

<211> 1368

<212> DNA

<213> Saccharomyces cerevisiae

<400> 284

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 aacttctatt tatttaatta tagagcaaag ccgattaccg gtggcttgaa tcccgacaat 1320  
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<210> 285  
 <211> 1929  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 285

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 gctttcactg aactgaaag attgattggt gatgctgcta agaatacaagc tgctatgaat 180  
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<211> 2067  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
  
<400> 286

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<210> 287  
 <211> 2643  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 287

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<210> 292  
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 <212> DNA  
 <213> Saccharomyces cerevisiae

<400> 292

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 294

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<210> 295

<211> 3351

<212> DNA

<213> *Saccharomyces cerevisiae*



<400>

295

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<400> 296

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 <211> 3342  
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 <400> 297

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 <211> 1548  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 298

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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<210> 301  
 <211> 3135  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 301

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 <211> 1131  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 302

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 303

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cgctactcag cgtaattga ttcagaagca cgattaatga tcaaaaattt gtgccatgaa 1320  
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ctactgaaga aaacagagtc ttctaaaagt ttactaaag gttattttgt gttgactaca 1560  
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3252

<210> 304  
<211> 591  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 304

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acactaaaag gcgctacgtg gggcgccaat tcttttgacg caaaactaga atttcagtgt 180  
aatgacaata tgaaacaaga cgagctgact agtcacacat gggccgataa aagtatccaa 240  
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gacggcgata atggtaagga tggtgacagc gaggggaaaa agccagctaa gaaggcaggc 360  
gggacttcgt ggttcacttg gttattccta tacgctctac tgtttacatt gatatacctg 420  
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attcaacgtt ctactcaatt tctcatttcc ttacctgaat tctgcaagga agtggtttcg 540  
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<210> 305  
<211> 771  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 305

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cagccgctag acgacccaaa cgtccaacaa gtgctccatc tcatgctcca ctatgccgtg 180  
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ttgcacatcg ccgcctacca gtcccgcggt gatatcgtag ccttcctcct ggaccaacca 360  
accatcaacg actgcgtgct caacaactcc cacttgcagg ccatcgaaat gtgcaagaac 420  
ctaaacatcg cgcagatgat gcaggtgaaa cgctccacat acgttgcaga gaccgcccag 480

gaattcagaa cagcttttta caacaggac ttcggccacc tagaatctat cctctccagc 540  
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ctgcacgaat tcgtcaagaa aagagacgtc atcatgtgcc gttggttgct tgaacacggt 660  
gctgaccctt tcaagagaga ccgcaaggca aactgcccac cgagctcggt aggaaagtca 720  
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<210> 306  
<211> 390  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 306

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gatcttggtt tcttactcct tttttggcgt ccagataagc accccattg ggcttcgcgc 180  
acctgtgttc tcagacagat ttttggcacg aagttgttgt tttccaatt ccaaattaaa 240  
cagtcttctt tctacaacgt tgatgttgac tatgggagaa gtctaatag accttttggt 300  
ctttcttgct ggagcaactt cggtcacaaa gctcttttcg gcattattat gtaccccgag 360  
tggtatatag gagcagcttc tcctatttga 390

<210> 307  
<211> 2067  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 307

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gaactctggt atagcgaatc atccgataat ccctctagct cattgtttgt ctctaatttg 180  
gataccaagg aaactttttt gaatgaagac aataatttgc agatttcttc cggactagac 240  
tattcttctg aaacgtgcaa tcaaggcagt aattacagcc aggatgggat attttatatt 300  
tcaaacgcaa aggctataaa cgcatacgga ggcattatca cgcaagggcc cgaggcacc 360  
atattagcta tgaaggtgtc cgactcaatg ccctatggag acggctccaa caaagttttt 420





tggcaaaaaga acaagatgat aatatag

2067

<210> 308  
<211> 2196  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 308

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aacgaccac catccacttt cacgcagtgg cttcttcaag atcccaaatt tcctcaacct 180  
catccagaaa gaaataagca ttcaccagat ttttcagcct tcgatgcgtg tcataatggg 240  
gcatcttttt tcaaactgct tcaagagcct gactcaggta tttttccgtg tcaatataaa 300  
ggacccatgt tcatgacaat cggttacgta gccgtaaact atatcgccgg tattgaaatt 360  
cctgagcatg agagaataga attaattaga tacatcgtca atacagcaca tccggttgat 420  
ggtggctggg gtctacattc tgttgacaaa tccaccgtgt ttggtacagt attgaactat 480  
gtaatcttac gtttattggg tctacccaag gaccaccggg tttgcgcaa ggcaagaagc 540  
acattgttaa ggtaggcgg tgctattgga tcccctcact ggggaaaaat ttggctaagt 600  
gcactaaact tgtataaatg ggaagggtgtg aaccctgcc ctcctgaaac ttggttactt 660  
ccatattcac tgcccatgca tccggggaga tgggtgggttc atactagagg tgtttacatt 720  
ccggtcagtt acctgtcatt ggtcaaattt tcttgcccaa tgactcctct tcttgaagaa 780  
ctgaggaatg aaatttacac taaaccgttt gacaagatta acttctcaa gaacaggaat 840  
accgtatgtg gagtagacct atattacccc cattctacta ctttgaatat tgcgaacagc 900  
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1000  
 900  
 800  
 700  
 600  
 500  
 400  
 300  
 200  
 100  
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 aaaaatagac aagaagaatc cggggaatgg aaatttgaaa gtgtagaagg tgttttcaac 2100  
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 atgtacagca gggcatatga aacacatacg ctttaa 2196

<210> 309  
 <211> 1587  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 309

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 aatagcaaag atggtgtaga aaagagaccc ctggaagatg taaagcaaat gattgacgct 120  
 ggaacaccag atgttggcca caaatctact gttgaaacta agccaaacgt tggatggcaa 180  
 gcctctcaca gtaatttggc tgcattacac gaaaaagagc agaaatatga aatggagcac 240  
 catcatgctc gtcataaact gcatcgtcaa gttattccgg attacacgtc tgcctcgacc 300  
 gcaatgttca gcgattgtat gttcaacgca gcaccagata aagtacgaag tctcagtacg 360  
 atgaagtctt ctggactctc gccaaaacac ccatttaacg tagtcgccac ctttaaagga 420  
 ccattcccgc agcatagtgt agaatcaaag cctctcgatg gtggatactc tgccaaagac 480

cattttccct catttaagat gttgcaagcc cagcagcacc cagcccatcg ccattacaaa 540  
 gacaacgaca agtacggtct taaatcacct tcccggctct tcgtgaagga caagaaaagg 600  
 ttggttcacc ggtttttgaa atccatggag ccttcttcgt ctgggcaatc taaggattcg 660  
 tctgcactgg cgccggcttt cgatccaata ttgccaatg ttatatctaa gccttccaag 720  
 cgacccacac atcattcgca ttcacagac gggagttcta gcacgcagac agatatatcg 780  
 ttacagagct tgctttacca tgatcttgaa agctcaccaa agaaacatgt ttcgccctca 840  
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 gaggattatt taatgaattt gaaacaggat ttgacaagg aaaaccccg aatcatttgag 1320  
 gcacgtttga gtgatacaat aaatacaaac gtggcaaaat tacaagattt agagaaaaga 1380  
 atggcttctt gcaaagacag gttggcctct aggaaggaag taatgaggaa aatggaaagt 1440  
 ttattgtctt tggagaattc cttaatgata tccaaaaaaa atgtaacatt cgcactctaa 1500  
 taccgcaacg aggcccttga tattgtcttt ttaattatca tcatcgtcat atgctatacc 1560  
 ttcaagcatc tagtatcgca taaataa 1587

<210> 310  
 <211> 435  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 310

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 taccaaatat ccgcttacca aacaagagca agacagaaag caagaacaga cgcacatacg 180  
 cctttggcac gcaattatat caaatcaatg gacctaatta gtaagaaaac caagacatca 240

ctgcttccta cgataaagag aacaatttgt aaaaaatgcc atcggttggt atggacccca 300  
 aaaaaactgg aaatcacatc cgacggagcg ctttcggtaa tgtgtgggtg cggtaccgtt 360  
 aaacgtttta atattggcgc cgatcctaata tacaggacct actcggagcg ggagggtaat 420  
 ctactaaatt cttag 435

<210> 311  
 <211> 3270  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 311

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 tcccctgaca atcaaatcgc ttctgtagct gagaaggctc ttagtgaaga atggattacc 120  
 gaaaacaata ttgagtatct tttaactttt ttggctgaac aagccgcttt ctccaagat 180  
 acaacagttg cagcattatc tgctgttctg tttagaaaat tagcattaaa agctccccct 240  
 tcttcgaagc ttatgattat gtccaaaaat atcacacata ttaggaaaga agttcttgca 300  
 caaatcgtt cttcattgtt aaaaggggtt ttgtcggaaa gagctgattc aattaggcac 360  
 aaactatctg atgctattgc tgagtgtgtt caagacgact taccagcatg gccagaatta 420  
 ctacaagctt taatagagtc tttaaaaagc ggtaacccaa attttagaga atccagtttt 480  
 agaattttga cgactgtacc ttatttaatt accgctgttg acatcaacag tatcttacca 540  
 atttttcaat caggctttac tgatgcaagt gataatgtca aaattgctgc agttacggct 600  
 ttcgtgggtt attttaagca actacaaaa tctgagtggc ccaagttagg tattttatta 660  
 ccaagtcttt tgaatagttt accaagattt ttagatgatg gtaaggacga tgcccttgca 720  
 tcagtttttg aatcgtaaat tgagttgggtg gaattggcac caaaactatt caaggatatg 780  
 ttgaccaaa taatacaatt cactgatatg gttataaaaa ataaggattt agaacctcca 840  
 gcaagaacca cagcactcga actgctaacc gttttcagcg agaacgctcc ccaaattgtgt 900  
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 gtatccatag atgatgatga tgcagcagaa tggatagaat ctgacgatac cgatgatgaa 1020  
 gaggaagtta catatgacca cgctcgtcaa gctcttgatc gtgttgcttt aaagctgggt 1080  
 ggtgaatatt tggctgcacc attgttccaa tatttacagc aaatgatcac atcaaccgaa 1140



tcttatataa tccgtgtttg tgcccaatac gctccatcta catatgctga cgtttgcata 2820  
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 cgttcttcaa cagagaatgc cagtgcagcc atcgccaaaa ttctttatgc atacaattcc 2940  
 aacattccta acgtagacac gtacacggct aattggttca aaacgttacc aacaataact 3000  
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 ttgggatttt tgccttctag tgatgctatg gcaattttca atagatatcc agctgatatt 3240  
 atggagaaag tacataaatg gtttgcataa 3270

<210> 312  
 <211> 351  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 312

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 ttagtttggg agagttggtt gacatattca attaaggaat catcgcttaa cgtagataga 180  
 aaagaccttg cattcaagcc gccggtcttt gctgttaaata gtgaatcatt gacactttgc 240  
 tggctaaggc aattgttttt atcaggtggt tccctattta tagagtattc caaatcgctg 300  
 tccaataaat ctacaagacc accatgttca cccatcgctg gatatgccta g 351

<210> 313  
 <211> 1146  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 313

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 gactctgctt ttaatgcagc ttactcacct catatgtatc cgaactctcc gtattatgaa 180  
 ggttcatgga ataccgggta tactcctcaa cttcatcatg tagccctca taatcaatat 240

ttccatccaa tacaaccgtc cactcagtag aattatacga gtcttcccaa ttatacagaa 300  
 aattacatac cgccagttca tcagaatata tcttatgcac cagcgcttaa ttacagaag 360  
 tggccatcat catattgtga aaacactcaa gccttaaaga atgataaaga ttaccaaaca 420  
 tcaattagtt atgaagatgt tgcaataccg accgtaaagg aaatacaatt gattgagaaa 480  
 aacagagggga aagatacgtt tatgaatgaa ataagtccag taccatcaag caaggatcaa 540  
 gcatctgcgg agcctacaga aatcccaaga aaggatcccg agctggcaaa ttctaagtc 600  
 gaagatgatac ataataactt agggctagaa gatgatgatac gtgatgagca gttagaatca 660  
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 gatgttaaaa agtggagaga agaaagaaaa aaaatgtggc tactgaaaat atccaataac 780  
 aaacagaaac atatgcaaga aatgggcata aaggaagatg aactgaagag ccaacctagc 840  
 attttcaaag agtcaaggaa agaaaagcaa ttatacaga gtattcaaaa tcaagtgcag 900  
 agaggaaatc cgaagattga cttgaacttg aaattgatac agcgagaatt cgcaaacgaa 960  
 aactcccaac ttttggactt tataagagaa ttgggagatg taggattact tgaatatgag 1020  
 ctatctcaac aagaaaaaga tgtactttttt gggagctctg aagataataa taaaaatcat 1080  
 tacaaccaa attacaaaaa caggaaacct aatctgagta gagccaactt tactagaaat 1140  
 aagtaa 1146

<210> 314  
 <211> 609  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 314

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 gctggagttt catatggtac ctttgggtac tgtaaaactt tgaattcttt ttctgtctct 180  
 cgagtacgtt taatatataa cacctcgaaa gaaatattac ctggcccttc tcttgaacgt 240  
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 gctacatgtt tgactttcat ttcttttgca cttcccttgg ttattatttt tctttttcag 360  
 actggaggaa ctaatgtctc acttattact tccaacgcta tattacacat attgacactt 420



ttatcaacta tttttgcatg caccgtgatc ctgttacttt gcatgcaccg tgatcctgtt 480  
acaatttcat ccttatacga cttggtgtgg ctggctaact gttccctgtt ccctctgctc 540  
gttattggcg tgcattttct cagttttagg tttgatacgt ctgcgcagtc agacagaaaa 600  
catagctaa 609

<210> 315  
<211> 345  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 315  
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aggtttaaag atgctactaa gatgtgtttt caccaccaa tgctttcctc gagtgtcgaa 180  
cctttcttct ctttgtatct caaccacgtt ccatatatgt ggtcgtgttt caactttttt 240  
tcttcgtctc cttttcctcg ttgcaccaa aattctccag aaaaaaagaa attcaaacgt 300  
gggctgccta ttagtagtaa atacacagac ggaagaagc gttga 345

<210> 316  
<211> 675  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 316  
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atcgacacgc agacgtccac gtttacgctc aagttccttc acacagaaac gggtcagaac 180  
atcgagaaga ttatctactt cattgacaat gacacgggca acgatacccg cactgctacc 240  
ggaatcaagc aaatcttcaa caagatgttc cgcacgctg cggagaagag gaagctgtcg 300  
ctcatccaga tcgacactgt tgagtacccc tgcactctag tagacttggt gatcctggta 360  
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ctggtcctta atcctgtggg ctccacctta gaggcgtggc tggatagtga tcttgtgctg 480  
cgactcatca ttgttgccga gttcctgact cagccctcg agacgtcat tttcgtcgtc 540

cctagggtca agtactaccg tgttcctggc gagttcgtgc ccgagtggct tcttctaggt 600  
 ctctcgaag gttacgggtcc tgcgagacgt ctagatacta aggctcgcac tcttggcgaa 660  
 gggtcagtta attag 675

<210> 317  
 <211> 1452  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 317

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 tccagactag atttgtatct gacaagaagg aggctggata cgtccatcaa tttacctaca 180  
 aacaccaaga ccaaggacca tcccccaat aaagagatgc tgaggattta cgtctacaac 240  
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 aagaagagga aacgcgattc gtcattgagc cttcctttga atctgcaaca acccgaatac 480  
 aatgatcaag atagcaccat gggcgataac gacaacggcg aggatgagga cagtgcagag 540  
 gcagaatcca gggaggaaat tgtagacgca ctggaatgga actacgatga aaacaacgtt 600  
 gtggagtttg atggtatcga catcaagagg caaggcaagg ataatttgcg atgcagtata 660  
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 ttgataggta tgcaaacggg ctccgttaat gacgcggttt attcgatcta caagtacatt 780  
 ttgatcaaca atctgtttgt tacggaacaa acagaggctc aagatgggtc caacgatgcc 840  
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gaaatcacia agcttgccctt gcagttgaac tctagtgtc aaaaatacca gtttttccac 1260  
gaactgtctt tgcattccaag agaaacgctg actcactact tatgggtcttc caagcaaaac 1320  
gagcttgtgc tgcagggcga ccaatacttc aatgaagatg ctgcaagaac gagtgacata 1380  
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ggaagactat aa 1452

<210> 318  
<211> 2157  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 318

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aattcgtttt acaagttttt gaactgggtt gacgaccgta cctgggtaccc cctcgggaagg 240  
gttactggag ggactttata tccctgggtt atgacgacta gtgcgttcat ctggcacgcc 300  
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caattgaaga aggatgatgc ccaaggtaga actttgaggg acgttggtga gttaaccagg 2100
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<210>      319
<211>      3660
<212>      DNA
<213>      Saccharomyces cerevisiae

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<400>      319

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ctgacgcttc tttctaaaat ctccgacgaa gccataaatg aaaacttgaa gaagagattt  180
ttgaatgcta caatctacac ttatataggt catgtgttaa ttagtgtcaa tccattccga  240
gacctcggaa tctatactga tgctgttatg aatgaatata aaggtaagaa tagattggaa  300

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gcagcccaag caagtttggg tgatggtttg gcgaatgcgc ttgctgctag ggccaataaa 3600  
atgagattag agagtgatga cgaggaggct aacgaagatg aagaggaaga tgattggtaa 3660

<210> 320  
<211> 1746  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 320

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aacttaattg agggcgaaga gcaagagggt agtgaagaag aaccttccta taccggcaat 180  
gacgatgaga cggagaggga aggtgaatac cattcgttgt tagatgcaa caattcgcg 240  
acattgcaac aagaagcgtg gcaacaaggt tatgactctc acgaccgtaa gcggttgctt 300  
gacgaagaac gggacctgct aatagacaac aaactgctct ctcaacacgg caacgggtggg 360  
ggagatatag aaagtcacgg acatggccaa gcaattggac cggacgagga agaaagacca 420  
gctgagattg caaatacgtg ggagagcgcg atcgagagtg gtcagaaaat cagcacaact 480  
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<210> 321  
 <211> 324  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
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 cgtgaatatg ctaattgggg cgctttccaa catgcgttta cgcggcgcgc tggagcttct 240  
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 cagtcagctg aatcatgcaa ttaa 324

<210> 322  
 <211> 2280  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 322

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 tctaggagtt cttctgcagc caatgtacgc tccggaacag gtgcaaatac acttactaat 180  
 ggcggcagta ctagaaagag acttgctgct actaattgta gaaatagaag gaaaaaatgt 240





tttatagaag actctcaaga atccgaaacg gtagtcatag agatcatcga tgatttgaag 1920  
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 ctttactcat gcctgttggc catcaactgt ttgaagaagt tgaaagagat acgttccagt 2040  
 gagcaagact catggaatgc acaattggat tttttcaatc acatcttcta cacacaacta 2100  
 tatcccgcat atgatctgcc gaaaaagctg caggaggata cggagctgga aacagaacaa 2160  
 gaaaaccaga tgttgaacca agttggaaat ataaactaca cgcacgattt ttcactctcc 2220  
 cacgaaattg acgatcttat cagagaactg tttggggtgg gcacacctca aaaactctga 2280

<210> 323  
 <211> 1023  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 323

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 attcttaaaa aacttagtga tttagaacaa aggaaaggaa gattttacgg gacagcaggt 180  
 tctccaacta ttgacaattt agaaaatgcc tggacgcatt taaccggcgg tgctgggaca 240  
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 ggtgatcata tcttgatgac tgatagtgtc tacgtgccaa cacgtatgct atgtgatggt 360  
 ttattggcca agttcgggtg tgaaacggat tattatgacc catcaatagg gaaggatata 420  
 gaaaaactag ttaagccaaa tacaaccgtc attttctctg aaagcccggg ttctgggacc 480  
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<210> 324  
<211> 336  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 324

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ataagatttt cggaataaaa aggtaaatgc agccaaaaat caaaataactt cagaagaagt 180  
cgtagcgagg actgctacgt ggaagcggat ttgaagatcc tttccagaac aagaaggagc 240  
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agcgtagtat ctcgagtttc cagaagttgc agataa 336

<210> 325  
<211> 2589  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 325

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gatttaaaga tatttagtga ttttatcagc agtttttcaa actataatgc atatacaaac 600  
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ataaactatc tacttgacaa atataataaa ttgggtaa at ctaatacaat ttctcaggcc 720  
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 gcgcatttat caccactttc tcaaccact tttgaagggt catattctgt gaacgctata 2520  
 caaaccceca gagatatacg aagggtggga tcagatcaaa agggcggtcg cagtgtcagt 2580  
 acctcataa 2589

<210> 326  
 <211> 2250  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 326

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 ctaggcgatg gatccaacac tgaatatgtg gttgatattt tcattgaagc cgctaaggat 180  
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 gacttaatac ttagcgggtc aaatttctac gaaaggaata accagaagca agactgtttt 780  
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agttcattgc ttcgaagttt cttagtgaac aatcactgga agacgaattt gacggatttt 2100  
tggcttaata gtgatgtaac agcgcccttg tggcaacgat ttttctattc ttcggatact 2160  
tcaaaagcta tgttgggtgg aactgaggtt gactactacg agctatacga atatccggcc 2220  
cgagaaggag aagtattacg tccaaactaa 2250

<210> 327  
<211> 375  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 327

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gataaaactac tcagttgtga atcggactgt ttcgcacagt atgtggatgg gcacttgtca 180  
catgttaatg aagtcataac acaatattct cctgaagcgc aaacagggca tgatgcagtt 240  
gaatcgcaaa caacacatcc gtcgctgcct ctagttgcag aagttgaaga ggtggctgtg 300  
cttgacaagg atgaacttgc agaagccttg gaggagctcg acattttttg tttgattttc 360  
agaaaacgaa cgtaa 375

<210> 328  
<211> 921  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 328

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aataatgaag aatacataat actctttggc ggaggtcggg acctgatact aggctccctg 180  
acaccttggt cgagctctca tttgtccaat caagccaacc cacaagacac cagtgagtat 240  
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ctctacaaa gcctcgcgcc acaggcctgc gaattcgacg gcgaagaagc acatgccttc 480  
accgtgcgca gcgtggaatt gactatcagg cccaaatatt caatatacga tcgccattat 540  
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ctggtaaaca attgtaacga agcactagcc gtatgcatgg acctgcacgg tgaggacgta 660  
caagatcaag accaggaaca ataccaagat ccaagcatgg cgtttgaggg tgcgcaagat 720  
ctcaacgcca cgtattctgg gcttgagac acccttcacg ggccgcccgt gtatcagaac 780  
gacgggctgg cagacgatct ggatggcgac ctcgttatgg acaacgtggc ctctaggggt 840  
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<210> 329  
<211> 1302  
<212> DNA

<213> Saccharomyces cerevisiae

<400> 329

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tcttctccct ctgaaatcaa cagttattgg aacaagtatt tttggaataa gctactatca 180  
tgggacagtg ttttttttat caagaacata acttccaaaa acggaaaacc tcaatttgag 240  
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aacgatagca tctaccatgc cttaagggtt ggagttgcaa tagaaaatgt tttattttac 360  
ttgtcaggta ttgttttata ttttctaaca aaaaaaattt tcagccaaaa tataaggcaa 420  
tcacagtttg ctagaactat cgctaaaaaa acatctctgt tgtttttctt aacgagtgcc 480  
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agatattgggt ttccttactc ctttatcagc atggcttget ttaccttagc atccttgaat 660  
cgttcaaact gtgttttgtt agggatttac tttatttttg accttattga actaacaag 720  
aacaggaagt ttgtaaaagc aatatgtttc ccactattat caggatcatt aatgttttct 780  
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atagtatgct tttttgcca tgtccaaatt ctgaatcgta tagcctcctt tttacccttg 1140  
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<210> 330

<211> 369

<212> DNA

<213> Saccharomyces cerevisiae



<400> 330

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aagatgtccc ttagttgctc tagagtgcac tcatcgacgt tttcttgga atgtttttca 180  
aagttagtag acatgttttt ttttttcttt cttgctgtgt atgttagaag gactacagtt 240  
tattctaacc taaacctaga gctaccgtca aatatacacg tgtattcact ggatttgcct 300  
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<210> 331

<211> 2142

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 331

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tacgatttca aaatgaacca gcagctggct gagatgcagc agataagaaa caccgtctac 180  
gaactggaac taactcacag gaaaatgaag gacgcgtacg aagaagagat caagcacttg 240  
aaactagggc tggagcaaag agaccatcaa attgcatctt tgaccgtcca gcaacagcgg 300  
caacagcaac agcagcaaca ggtccagcag catttacaac agcaacagca gcagctagcc 360  
gctgcatctg catctgttcc agttgcgcaa caaccaccgg ctactacttc ggccaccgcc 420  
actccagcag caaacacaac tactggttcg ccatcggcct tcccagtaca agctagccgt 480  
cctaattctgg ttggctcaca gttgcctacc accactttgc ctgtggtgtc ctcaaaccgc 540  
caacaacaac taccacaaca gcaactgcaa cagcagcaac ttcaacaaca gcaaccacct 600  
ccccaggttt ccgtggcacc attgagtaac acagccatca acggatctcc tacttctaaa 660  
gagaccacta ctttaccctc tgtcaaggca cctgaatcta cgttgaaaga aactgaaccg 720  
gaaaataata atacctcgaa gataaatgac accggatccg ccaccacggc caccactacc 780  
accgcaactg aaactgaaat caaacctaag gaggaagacg ccaccccggc tagtttgcac 840  
caggatcact acttagtccc ttataatcaa agagcaaacc actctaaacc tatccacact 900

ttccttttgg atctagatcc ccagtctgtt cccgatgctc tgaagaagca aacaaatgat 960  
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<210> 332  
 <211> 3108  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 332

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tttgcttaca agtccttttc tcaacccgat ttgctacacc aagatctaaa aaaatggtct 180  
 gaaaagcgtg gtaacgaatc acgtgggaag ccatttttcc aagagctgga tatcagatct 240  
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 gctccagggg tttcgtgccc atacttcatt aactctttga aaaccgtctc tcatgatggg 360  
 aagtttcttt tgaatggttg tgctttaaac tacgacaatg ctaccggctc tgtcaccaac 420  
 gattatgtaa ccgcattgga tgctgcttcc aagctgaagt atgggtgtcgt gactccgatt 480  
 tccgctaacg aggtacaaaag tgtcgcctta ctggcattgg cgattgccac tttcagtaat 540  
 aactccggag ctatcaattt atttgacgga ttaaactact cgaaaaccgt cttgccgttg 600  
 gtcgaatctg ttctgagggc atctattttg gcaaaactat ccaaagttat tgcaccagat 660  
 gctgcctttg atgatgtctt ggataagttt aatgaattga ctggattgag actacataat 720  
 ttccaatact ttggtgctca ggatgctgaa actgtgttta tcaattatgg gtcttttagaa 780  
 tccgaattgt tcaactctgc gattagtggg aataattcca aaatcgggtt aatcaacgtc 840  
 agagtgccat taccttttaa cgttgctaag tttgtcactc acgttccatc cactaccaaa 900  
 caaattgttg ttataggcca aactttggat ggttcttcgc cttctttctt gagatctcaa 960  
 gtttcagccg ccttatttta ccacggccgc acctcaatta gcgtttctga gtacatctat 1020  
 caaccagatt tcatttggtc cccaaaagct gtcaaatcaa ttgtatcgtc attcatccct 1080  
 gaattcactt acaatgccga ttcattcttc ggcaaggat tcatttattg ggctctgat 1140  
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 ccaaagcaac cagttattat gagtgggtta ggtactgggt tggcaccatt caaggccatt 2700  
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 tacattcaag atcgtatcaa agagaatttg gatgaattaa aaactgcaat gattgataat 2940  
 aaaggttcat tttacttggt tggccctact tggccagttc cagatattac tcaagctttg 3000  
 caagacattc tggctaaaga cgccgaggaa agaggcatca aagtcgactt ggatgccgca 3060  
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<210> 333  
 <211> 1923  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 333

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 tctgcagacg ggaagtacca cataatagat cacgagtatg actgtgtggt aatcggtgcc 180  
 ggtggtgccg gccttagagc ggcccttggg cttgccgagg cgggctacaa gactgcttgt 240  
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 gctctgggaa atatgcacaa ggataactgg aaatggcata tgtacgatac tgtgaaagga 360  
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 atcattgaac tggaacacta tgggtgttctt ttttcaagaa ctgaaaacgg taagatctac 480  
 caaagagcct ttggtgggtca gaccaaggag tacggtaagg gtgctcaggc ctatagaaca 540  
 tgcgctgtcg cagacaggac aggacatgct cttttacaca cgctttatgg ccaagcttta 600  
 agacatgaca cacatcttctt tattgagtac tttgcctctg atctgttgac ccataatggc 660  
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 cacaagacca ttattgccac tgggtggctat ggtagagcat acttctcttg tacctctgct 780  
 cacacatgta cgggtgacgg taatgccatg gtttcgctg ctggtttccc cttgcaagat 840  
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catacattat cctggcaaaa ggacgtcgct gccccagtga ctttgaaata cagaagggtt 1860  
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taa 1923

<210> 334  
<211> 447  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 334

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acttacgttt ctggttttaga attgacacgt aaagtctacg aaagaaaacc cactgaaaca 180  
accattttga gtgcaagaac tttcgggtact tggaccttta tttcctgtgt tatcagattc 240  
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gttgccctat tccacttcgg ctctgaatta ttgatcttta gaacttgtaa gttgggaaag 360  
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gaatactaca ctggtgttgc ttggtaa 447

<210> 335  
<211> 2667  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 335

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gttggcgaca tcagtaggga tgacaacata caaatttact ggagggatcc tacattat 180  
gcacaatata taactgattc taatgtggtc gctcaagaac aagccattgt tgccttgaat 240  
agtttaatag atgcctttgc gtcacgtctt ttgaaaaatg cacataatat tactttaata 300

tcaacatgga caccattgct tgtggaaaag ggcttaacgt cctcaagggc tacaacgaag 360  
acgcagtcga tgagctgtat actctcacta tgtggctctg atacatctat tacgcaatcg 420  
gtggaattgg taataccctt tttcgagaaa aaactgccaa aattaattgc tgcggcagcc 480  
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gaattggaaa agaaaagaag ccaagaggag gaagccagga aaagaaagtc catattatcg 840  
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aagttaaaaa gcacctcgca aaattattcc aacttattgg ggatatatgg ccatattatc 1140  
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<210> 337  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 337







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 tcatttggtg gagatttaat tgaaagctat tggactgcta ttcaaagtac catgaacttc 2880  
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 ggccaagtgg cttccatagt ggccactatt ggttacagaa tatacacaac taatgagacc 3000  
 actaaaacgt atctacagga acacatcaaa gaaaagaacg caaaggtttc tttgattaaa 3060  
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 gcaaagtgtt tgaaggcaaa aattgcagaa aagatcaaaa ttttggaaac tcatgacgtt 3300  
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<210> 339  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 339

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 aggaaaactt cactggtct tcaactggaa caattggcca acacaaattt attaaccata 180  
 agaataaaat ggagttaca agaagaagaa gatgatcact gcaactctag aataaccgat 240  
 caaataatgg acacaatata gcactacaaa ggtatctccg ttaacaactc tgatacagaa 300  
 acatatgaat ttcttccgga tacaaggagg ttacaggttc tgaacaaaa taaagacatc 360  
 tatctttacg agcatggaag tcaagagtat gagaaatctt acaaagataa cgaagaggaa 420  
 gatgattgga gatacgatac cgttttgcaa gcacaattca agtaccocaa gtcattagaa 480  
 aatgcatgta cagatatctc ggaattactc aagagcgaac ctattggtca gcatattgat 540  
 aaatggtcta tcggtgtgaa caagcatgca ctaacctatc ctggaaatat ttttgcggg 600  
 ggaatagcaa agagcctttc tattggtgaa ctaagtttct tattttcaaa atatggacca 660  
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2346

<210> 340  
<211> 2193  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 340

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gtctccataa accattggca gttgcgggat tgtatcaaac caggctccat gaaccagagt 180  
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gaaaaaacgt ctacaaaagg ttctaggaca acgggctcgt atatctccaa aaatttacat 360  
gttccttccg agaaactggg agagttcaac ttttaagccga gatgtttcac ggaattaaat 420  
gggttgactg tttgtgggtg tctaatcggg tctgatgata agggattccc ttctaattgg 480  
aaccgtttag ctcaagatgc aaatatttcg ttgccgccac cttcccaacc gattaatatt 540  
tccaaaaata ttagtttccc tataaattca cattattcaa atcctaatat ctggaagggc 600  
atcgtggagt ttataatca agaaacggac acaatgatga cttttacgtt gggccagttc 660  
atcaataatt gtgtcacact ttatgacagg gccagtatgc agtttgatct ttttgcatgc 720  
aacaatgatg gtcattctta ccaatgcgat gtaagtaata gagatgtaac actagtgaag 780  
cgttatgcgg atttaaaatt tcctttgaat aacgcttctt tgtcacatga tgggtcaaacg 840  
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caattctcct tacattacga taatcatcct tcctggggta gttctgtcaa tcgtgttaga 960  
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tatggcctag atgatttatt attcatttct gaacatcagg gcagagtaca cgttgtagat 1260  
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<210> 342

<211> 2295

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 342

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tggccaaatt tacccttcca ggccttcct gatggttctc actcgtttga agaaacattt 180  
acttatttca cattactata tgatgaaaga agacaaagga gccctcccaa cggggcaact 240  
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 catgataaag aaaatgaaca gaattttgag ggtaatgcag aaaattTTaa tgaggacgaa 2100  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 gaaactgctc caaataaaga ctccaacact ctatttcaag aatgttccca ttactacaga 240  
 gattcctctc tagatgggtg ggtatcaatc accgcgcgatg aagctagtga gttaccagcc 300  
 ccacaccatt actatctatt aaacctgaac ttcaatagtc ctaatgaaac tgactccatt 360



gtattagcat ctgatcggtt accatataaa aattatgact acgaccgcgt atttggcgct 2040  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 344

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 <211> 1050  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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<210> 346  
<211> 2523  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 346

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attaggatat ggtccataga ttccattttg cgttgcatgg aactagaatc tctaactcct 180  
gagatacctc ttccgcaaga cttgcagatg ccgttatgta gcatgagtag gcataccggc 240  
tcgataacat gtgtcaaatt ctcaccggat ggaaagtacc ttgcaagtgg atccgatgat 300  
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caagacattt gctgggccc agactccagc atactagtga cagttggttt agatagatct 480  
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gataaaaaca tcgactctgt tgtggcaaca gctgggcaag ataaatcctt agcgggtctgg 1020  
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<213> Saccharomyces cerevisiae

<400> 347

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<211> 1020  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 348

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<210> 349  
 <211> 1050  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 349

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 ctccctgaaa aactttcaaa acagtttact tttaatgtag acgacataaa atttcataag 180  
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<210> 350  
 <211> 2553  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 350

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[illegible]

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Age	34.5	10.2	18	65
Gender	50%	50%	0	100
Marital status	65%	35%	0	100
Education	12.5	2.5	8	16
Income	3500	1500	1000	8000
Health status	75%	25%	0	100
Stress level	60%	40%	0	100
Life satisfaction	70%	30%	0	100
Work-life balance	65%	35%	0	100
Family support	70%	30%	0	100
Community involvement	60%	40%	0	100
Personal growth	65%	35%	0	100
Overall well-being	70%	30%	0	100

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 <213> *Saccharomyces cerevisiae*

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 <213> *Saccharomyces cerevisiae*  
 <400> 383

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<210> 384
<211> 369
<212> DNA
<213> Saccharomyces cerevisiae

<400> 384

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<400> 385

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caggcgacag cttccatgtc gatcgttgcg ttaccgtcta gcttccagga gagcaatagc 180
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actaccaccg aaagtaccga ctccaacact agtgctacta cactgctag caccaactcc 600
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 <400> 386

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 aatgccgatt tcattttcaa tgaacaagaa atggatgttg aagaccagga ggatgagggg 180  
 tctgaatcag ataacagtga agaagatgaa ggcgagaacg gtgaattaga ccatgttaac 240  
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 500  
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 300  
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 <211> 411  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 <213> *Saccharomyces cerevisiae*

<400> 388

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<400> 390

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 <211> 2958  
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 <213> *Saccharomyces cerevisiae*  
 <400> 391

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 gatgacgaag agttgccgcc cttacctgtg gaggcacaaa caggaaacga tgggtccaggt 2880  
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 cttggtctca ataagtaa 2958

<210> 392  
 <211> 168  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 392

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 tatgtttccg atatcagagc tcatttggct gaatactact ctttctaa 168

<210> 393  
 <211> 2454  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 393

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 gggatgaaaa aagcaaaggc ccctgcactg cctggacacg ttccacctcc accagttcca 1860  
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<210> 394  
 <211> 1788  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 394

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 gaggaggcct tgaaaaccgg ggaggagccg gaattgaagc ttgatatcag tgccaatcgt 180  
 tacgatttgc tttgtatcga aggtatttca cagtcgctga acgaatactt ggaacgtaaa 240  
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 tcttacgctt cttttattgc cttgcaagat aaattacatg ccaatctatg tagaaacaga 420  
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 gctctaccac caaaggacat caagttcgta ccattgaatc aaaccaaga gtttactggt 540  
 gacaaattga tcgagtttta taaatctcca gaacagaaaa acaacatagg gagatacggt 600



cacattattg aggatttctc agtcttccca gttattatgg acagcaaaga tcgtgtttgc 660  
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 ttgattgata taaccgccac cgataagacc aaagccgaga tcgttttgaa catattaact 780  
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<210> 395  
 <211> 2640  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 395

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 ggcggtgctg ctggatcaca gcaaattggc ggcttcagaa ggctcgagttt caccacggca 180



aaaattcaaa aaaatacttt ggttgatcaa tttaaaggta acatgaaaca gttatctcta 1860  
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<210> 396  
 <211> 2040  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 396

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 ccagcaggaa cgccaatgt accaacacgt agaccatac ttaaagcaaa gacgatgact 180  
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 gataacaaaa cttctacaaa tccctcccca ctcgaaaaaa atgagcacga ggggtgctgag 480  
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gaggcagggg atagaagcca ttttgaagaa aagctcattc ctggagatat gaaagtacag 660  
gtagatgtta gtaaggacgt agaagaaggc agcctcaatg ctctgcctcc atctggaatc 720  
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<210> 397  
<211> 2841  
<212> DNA

<213> Saccharomyces cerevisiae

<400> 397

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aaattcaatc cgaactccac caccttagct tctatcgaag acagttttac ccagttcaag 180  
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1000  
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 700  
 600  
 500  
 400  
 300  
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 100  
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 gccagtttaa cgagctctaa attacaatct ttcttaaaga aaaaaattgt cattccccctt 2040  
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 <211> 1107  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 398

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 aatgcgccct cccaccagtc gaactaccac cccattaca atcacatgaa atacaacaac 180  
 actggtagct attactatta caacaacaac aataacagca gtgtaaaccc acataaccaa 240  
 gctgggtctac aatccattaa cagatctatt ccatcggccc cgtacggggc ttacaaccag 300  
 aacagagcta atgacgtacc atatatgaat acccaaaaga aacaccacag atttagcgct 360  
 aacaataatt tgaaccagca aaaatacaag caatatcccc agtatacgtc caatccaatg 420  
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<210> 399  
 <211> 1875  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 399

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 caatcacacc atatgttcaa taagcatggt caactcagaa gcggagattc tttatccttg 120  
 ttgagctgct tgtcctgtct ggatgatgga actttgagct ctgatggagg ttcttttgat 180  
 gaggatgatt ccctggaact gttgcctctt aatactacca ttccgttcaa cagaattttg 240





tttgaatcca tgtag

1875

<210> 400  
<211> 858  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
  
<400> 400

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attctaagaa ctaacaatcc agaacttgct ggtgatagaa gtggtccaaa gttcagaatt 480  
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agtattaaca ccgaattgaa gagagaacag tcaaacagac tgttctttat ggtctgtaaa 780  
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<210> 401  
<211> 951  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
  
<400> 401

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 gatagaaggg agcttcatga atctctgatt tttgctatga aagatgctga tttataccaa 1260  
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<210> 411  
 <211> 1491  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 411

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 ggtaagaaag ttcttatcgt agaacgtgac tgggctatgc ctgatagaat tgttggtgaa 180  
 ttgatgcaac caggtggtgt tagagcattg agaagtctgg gtatgattca atctatcaac 240  
 aacatcgaag catatcctgt taccggttat accgtctttt tcaacggcga acaagttgat 300  
 attccatacc cttacaaggc cgatatccct aaagttgaaa aattgaagga cttgggtcaaa 360  
 gatggtaatg acaaggctctt ggaagacagc actattcaca tcaaggatta cgaagatgat 420  
 gaaagagaaa ggggtgttgc ttttgttcat ggtagattct tgaacaactt gagaaacatt 480  
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gatcctgaat tcatagagtc gtacatttac ggagaaacat acatgacgga ggaggaagaa 480  
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gctcactggt gggctacgct atggaaaccg cgtgccacac tggtagagtc tgtgtctacg 1260  
atgtggtcaa cgacaagggc tggagtgtaa 1290

<210> 414  
<211> 1023  
<212> DNA  
<213> Saccharomyces cerevisiae  
<400> 414

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tcaggcgatg ctaagatggc tatttttgac gagctcttac agatgccaga aacaaagctt 180  
gttgcagggtg gtgctgctca aaacactgct agaggggcag catacgtttt gggcgccggc 240  
cagggtggtgt acttcggttc cgtcggttaag gacaagttca gcgagagatt gcttaacgaa 300  
aacgaaaaag ctggtgtcaa gtctatgtac caagttcaaa atgatattgg taccggtaag 360  
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tacatcggtg gtttccactt gaccgtgtct ccagacgcta tcgttaagtt gggccaacat 540  
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ttcaaagacg cattggccag agttttgcct tatgctaccg tcatcatcgc taacgaatcg 660  
gaggccgaag ccttttgoga cgcttccaa ttagactgtg ccaacactga tttggaagct 720  
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gtcgaaccaa cagtggctgt gtcctccaag ggtaccagca catatccagt caaacctttg 840  
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gctgggttga ctaaagggtga agatttggaa acctctattg acatgggtca atggctagct 960  
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tag 1023

<210> 415  
<211> 2535  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 415  
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ctaaaaatta ataatcctgc aattgatacg gtaacattga acaccgtcga taccgacatc 180  
cattctgcaa aaatcgggtga tgtcacatct tccgagatta tctctgaaga ggagcaacaa 240  
gtcaccacat ttgcgtttcc taaagggacc atgtcttctt tcaagggtaa tgcatttttg 300  
gatataaaat ttactggtat tttaaagat aatatggctg gcttttacag agccaaatat 360  
gaggacaagt taacagggtga gacaaaatac atggctacta ctcatgga acctactgat 420  
gctagaaggg ccttcccatg ttttgatgag cctaacttga aagcctctt tgctattact 480  
ttagtttcag acccatcttt gacacaccta tccaatatgg acgtcaagaa cgaatacgtc 540  
aaggatggaa aaaagggtcac tttgttcaat acgacccta aaatgtcaac ttaccttggt 600  
gcttttattg ttgctgaatt gaagtacgtt gaatccaaga actttcgtat tcctgtcaga 660  
gtttacgcca cccaggtaa cgaaaaacat ggtcagtttg ccgcggattt gactgccaag 720  
actttggcat tttttgaaaa aacttttggc atccaatatc ctctaccta aatggacaat 780



ggtacctctg gctttacatc aatgcaaaaa atagatgaga ttaagaaatt ctttgccact 2460  
 aaatccacga aaggtttcga tcaaagtttg gctcaatcac tggacactat tacttcgaaa 2520  
 gcgcagtggg gttaa 2535

<210> 416  
 <211> 2259  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 416

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 aagcaggtag atttgtccac tgaagtgcaa tttgtgtccg atttattgat tgatgcgggt 180  
 gcgtcaaagg ctaaagttaa agaactatcg gaaagtattt tgaagcaatt gactactcaa 240  
 ctaaaggaga acgaagccaa attggaattg accggtgata cgtccaagag attacttgat 300  
 attaattgtct taaagagtca taacagtaaa tccgatatca acgtctcatt aagcatgctg 360  
 ggtgtgaacg gtgacatcga acatactggt agaaagatgg aaacaagagt tgatttgaaa 420  
 aaactggcca aggctgaaca aaagatcgca aagaaagtcg ccaagagaaa taacaaat 480  
 gttaaatacg aggcttctaa attgatcaat gaccaaagg aggaggatta cgattctttc 540  
 tttttgcaaa tcaacccttt agaattcggg tcatccgctg gtaaattcaa ggatatccat 600  
 attgacactt tcgacttgta cgttggtgac ggtcaaagaa ttttgtccaa cgcccaattg 660  
 actctaagtt ttggtcacag atatggtctt gtgggcaaaa atggtattgg taaatctact 720  
 ttgttaaggg ctctatctag aagagagctg aacgtcccca aacatgtttc gattttacac 780  
 gtggaacaag agttaagagg tgatgatata aaggctttac aaagtgtgct ggatgcagac 840  
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gaaaaaattg tagaacagag ttttgacaaa cgggatttga ttctatttag tgcattgggt 1980  
tctctatacg ataatgacat aattgaggag gatgtcattt ataatgggtg ggataatggt 2040  
tctactgacc ctcgctatga tgaagtcaag aaattaactg taaagtgggt tgagtgggta 2100  
cagaatgctg acgaagaatc ttcctcagaa gaggaataa 2139

<210> 418  
<211> 336  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 418

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ataagatttt cggcaataaa aggtaaatgc agccaaaaat caaaatactt cagaagaagt 180  
cgtagcgagg actgctacgt ggaagcggat ttgaagatcc tttccagaac aagaaggagc 240  
cgaaagctgc caggaactgt tcctgatttt ttaggaaaac aattaatagg tatctcgtct 300  
agcgtagtat ctcgagtttc cagaagttgc agataa 336

<210> 419  
<211> 2460  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 419

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atagggggta gcaccccgac caataaactg aaattttatc catattcgaa caacaaattg 180  
acaagaagta cggggacctt gaacctgtca ttaagtaata cagctttgtc agaggctaac 240  
tccaaatttc ttgggaaaat tgaagaagag gaagaagagg aggaagaagg caaggatgag 300  
gaaagcgtgg attctcgtat taaaaggtgg tctccgttcc atgaaaatga aagtgttact 360  
actcctatta caaaaagatc tgcggaaaaa acgaacagtc ctatttctct caaacaatgg 420  
aaccagcgat gggtttccgaa aaatgatgct cgcactgaaa atacatcctc atcctcttca 480  
tatagcgtcg ctaaacctaa ccaatcagcc tttacgtctt cgggcctcgt atctaaaatg 540





atcaacaacc ctaatatgaa taatggcaac gataataata atgtcaatac tgccgctacc 2220  
aagaatcgtc ttatttttgca taaaagttct aaaattcccc catgggtacc gaaatttctt 2280  
attgatggtg aatcacttga gagaatagta cgatggatga tagagcccaa ttatgagaga 2340  
aggcccacgg caaatcaaact cttacaaact gaggaatgcc tgtatgtaga aatgacacgc 2400  
aatgcaggtg ctattatcca ggaagacgac tttggaccta agccaaaatt ttttatatga 2460

<210> 420  
<211> 1668  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 420

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ttaatcttcg ttggtgctac cattggtggg cttttattcg gctatgatac tgggtgttata 180  
tcaggtgttc tgctttcttt aaagcccgaa gatctatctt tggtagtttt aacggatggt 240  
cagaaggagc tgataacttc cagtacaagc gtcggatcat tttttggctc tattctggca 300  
tttccttttag cagataggta tggaagaaga attactctcg caatctgctg ttcaattttt 360  
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ttgctgggtg ggatcgctgt tggagtgtct gcccagtgcg tccctctatt tctgagtga 480  
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gggtcaattgg tttcctatgt gattgcgtcc cttatgaaag agattgataa ttcatggaga 600  
tatttggtcg cactttctgc tattccagct attctgttcc tttcaatact ggattttatc 660  
cctgaatcgc cacgatggtc catttccaag ggagacattc tatataccag ggattctcta 720  
agaatgctat atcctactgc atctacatat catgtgaata gtaaaattaa gcagctgata 780  
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ctatcggcgt tgcgaggccc agctccaaac ggtgccctgg catctaaca aaagaaaaga 1020  
cacagaatgg agccacgcac aataagagcg ttaatagtag gttgcatgtt aatgtttttc 1080

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 aatataaaaa accctctact gcctccaatt ttgattgcct caacgaactt catattcact 1200  
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 ttcgtatatt tttggtatcc agaagtcaaa ggtttgtcac tggaagaggt tggaaggggt 1620  
 tttgataatg gaattgatgt tcattatgtc tttcgtacct accattga 1668

<210> 421  
 <211> 2493  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 421  
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 aactacgagg ttagttcact attttatggg acggaacggt ttcagattca aaaccatttc 180  
 caatcgctcc tagatttagt agaccttaat aatgaaaatg gtggcttggt attcgacttg 240  
 ataataatga gtgcgtcttc actgcaagaa atcccgagg tattgagaga tataaaacct 300  
 atgatgaata agacaacaaa gatactatct gaaagtagcg ggtttatata tttggagcca 360  
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 attcctatct tgaatacatt tcagaaattg ttccaaaaac tgtttcctag ggacgttgtg 600  
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 caaatatgtt tcgatccatt attaataata ttagaagaga agaatccatc aaccttggat 720  
 gaccacgttt tggccaaacc tcttatttca ggtttactag gcgagagctt attaataata 780



aatataagta caatgggaga tgaaagccgc aaagaggacg ttaaggaaaa aaagaagaaa 2460  
aagttcagtt tctttggaaa aaggaaaaag taa 2493

<210> 422  
<211> 1731  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 422

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caccagcac cacctcatga ggcaaagcgt ccacaccatg gaaaagggtcc catgcactca 180  
cccaaattgtg agaagattga accattaagt ccatcattca aacattccgt cgacacaatt 240  
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cccactgtag tccaagacaa aaacccaac ccgcagatg atccggattt ctataagcat 360  
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ttggagaaag tcaatgagct gggctcttcta tacacatggg aaggttctga tcttgatcta 480  
aaaccattat tgttaatggc ccatcaagat gttgtacctg taaacaacga aactttatca 540  
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aaccaatcac atttgccgag cttaataaga acaacacaag ctgttgatat catcaatggg 1200  
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 gatttactga agacattgca ttcggttaat gaacacgtgg atgtcccagg tcatttatct 1680  
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<210> 423  
 <211> 2199  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 agaaaatggt ggtactacgt gaaggtaagg cccggtttaa aggaattttt cgctaaagtg 780  
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aatgggtctc taacaactaa atcattagct aaacttttcc ccactgacca gtcaatgggtt 960  
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 ccttataact tcttcgttgg tgtaggtgac attaattcta atttcttgcc caaacaatct 1080  
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 ctgacggata ttatggataa cgaaaaaaaa ctacaagaaa agatagataa agaagttaaa 1200  
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 tccaaagaag agttgaccaa gaaactagaa tattcagcat ctttagaagt ccaacaacaa 1320  
 aatcgaccct tggccaaact acaaaaacac ctgcatgac aaaaactact agttgatgac 1380  
 gatgatgaac tatactactt aatgggtacg ctatcaaaca ttcacaaaac ttattatgat 1440  
 atgctctcac aacaaaatga gccagaacca aatctgatgg aaatcatacc aagtttgaag 1500  
 caaaaagtct tccaaaattg ctattttggt ttttcaggat taatacccct cgggaccgat 1560  
 attcaaaggc cggacatagt gatatggacg agtacatttg gtgccacttc cactccagat 1620  
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 gcgaaaaagt tcaaccaca gattaaatt gttcaccag attggatatt tgagtgtttg 1740  
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 acccaggaac aacagcatat gttgacatca caagaaaatc taaatttatt cgctgctggt 1920  
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 gaagatgacg accacgacga cgaaagtgat gacgaaaaca actcgggaagg cattgacaga 2040  
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 tctcaagatg gtcctgtaca acataaagga gaaggggatg acaacgaaga cagcgattcg 2160  
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 <211> 936  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 424

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 gttatggagg ggttcaagta ccattttcct gaaaaggacg ttgtaaccgt gtggtccgcg 780  
 ccaaattact gttatagatg tggtaatgtc gcgagtgtaa tgaaggtcga tgaggatctg 840  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 425

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 agatcatcta atctcaaaag aaaggcttat tcgaggcccg tttctaata taacggttac 360  
 aacggaaaca gtaacagtaa ccaaaacaat actaataacg gaatggtaac accctcgaac 420  
 tattatagaa tggggagaaa ttctttctcg aggaacaaca acagtacgag gaatgttact 480





gtacagttct atgcaaattc atttattgat ccaatagtgt tacatgggaa tatgttgaga 2160  
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 <211> 345  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 426

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aatgccggtg gtgttggtga attggaaaag aaagcagagg ctggtgtgca aggtgaatta 240  
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<210> 427  
<211> 2841  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 427

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gattccaccg aatcactaat tcatggaggc gcagaaaggt atattgttaa cgctttaaag 180  
cctatagaat taaataaaac tgaaggcttt ttcgaagacc cgccgttcca tcttccttct 240  
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ccatccaaag ttgaccatat tgattttggc agaatacccg ctgtaccttt tagcctaagc 2760  
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 ttaagtcgta aacgtcaata g 2841

<210> 428  
 <211> 1254  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 428

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 agggcccagg cacaggagca ggatgacaag atcggcacca tcaacgagga ggacatcttg 180  
 gctaaccaac ccctgttggt acagtccttc caggacagac ttggctcgct ggtggggccag 240  
 gacagcgggt atgtgggggg tcttcccaag aacgttaagg aaaagctgct gagcttgaag 300  
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caagacgatt ttgctggcag gccggaacag gtcctgaat gcaagcagtc ataa 1254

<210> 429  
 <211> 1362  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 429

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 caacggccca tggctcctcc acctaaccag cagtatggac agcaatatgg tcagcaatat 180  
 gaacagcagt atggacagca atatgggcaa caaatgatc agcaattcag tcagcaatat 240  
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 ttccagcagg aacaggcaaa ggcacaatta agcaacggct acaacaatcc taatgtaaac 360  
 gcatccaata tgtacggtcc accccagaat atgtcattac ctccaccta aacacaaact 420  
 attcaaggta cagaccaacc ttatcagtat tctcaatgta ctgggcgtag aaaggctttg 480  
 attatcggtg taaactacat aggttcaaaa aatcaactgc gtggttgat caatgatgct 540  
 cataacatct tcaacttttt gactaatggg tacgggtaca gttcagatga cattgtcata 600  
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 gcaatgtccc acgccttcat caaggttatg actttacaac cacagcaatc atatttatct 1260  
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tcgtcacacc ctattgacgt aaatctgcaa tttattatgt ag 1362

<210> 430  
 <211> 1164  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 430

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 gctgtctacg gtgaagatgt tgacaccgtt aggctcgaac agaccgttgc ccgcatggct 180  
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 ctaatgggtg gtagagtctc gttccactat caagtcacca gagatacttt ggaaaaagtc 1020  
 aaattggcca tctccgaggc cttcgactat gctaaagaac atcctttcga ctgtaacgga 1080  
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 gaaataaaaa cctacaaata ctga 1164

<210> 431  
 <211> 2469  
 <212> DNA

<213> Saccharomyces cerevisiae

<400> 431

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gaaatagagg cttctctgag gaaagttttc caagatttca aagaaactca agatgtctca 240  
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<210> 432  
 <211> 2403  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 432

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 aagctgggtt ggaccaaggc ttctcctcgc aacgaggttc ctaagcaagt ggcaatcaaa 240  
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 tga 2403

<210> 433  
 <211> 2241  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 433

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<400> 443

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<400> 445

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 <211> 2532  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 447

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2532

<210> 448  
<211> 1359  
<212> DNA  
<213> *Saccharomyces cerevisiae*  
  
<400> 448

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actttgaaat ccacgagttc gacaaactct ccagattaa 1359

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 <211> 369  
 <212> DNA  
 <213> Saccharomyces cerevisiae

<400> 449

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<213> Saccharomyces cerevisiae

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<210> 452  
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<212> DNA  
<213> Saccharomyces cerevisiae

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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 <213> Saccharomyces cerevisiae

<400> 464

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 <213> Saccharomyces cerevisiae

<400> 465

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 <213> *Saccharomyces cerevisiae*

<400> 467

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 <211> 1449  
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 <213> *Saccharomyces cerevisiae*

<400> 468

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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 469

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324

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 <211> 363  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 472

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<400> 473

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 <211> 1692  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 476

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 <213> *Saccharomyces cerevisiae*

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<213> *Saccharomyces cerevisiae*  
<400> 478

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<210> 479  
 <211> 2109  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*



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<212> DNA  
<213> *Saccharomyces cerevisiae*

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<210> 481  
<211> 2115  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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<211> 3195  
<212> DNA  
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<400> 483

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<213> *Saccharomyces cerevisiae*

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<211> 1335  
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<213> *Saccharomyces cerevisiae*

<400> 486

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<213> *Saccharomyces cerevisiae*  
<400> 487

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<211> 2145  
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 <213> *Saccharomyces cerevisiae*  
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<210>      490
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<213>      Saccharomyces cerevisiae

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<210>      491
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<212>      DNA

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<213> Saccharomyces cerevisiae

<400> 491

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<210> 492

<211> 1377

<212> DNA

<213> Saccharomyces cerevisiae

<400> 492

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aagttcgaaa aggaagccgc tgaattaggt aagggttctt tcaagtacgc ttgggttttg 180  
gacaagttaa aggctgaaag agaaagaggt atcactatcg atattgcttt gtggaagtgc 240  
gaaactccaa agtaccaagt taccgttatt gatgctccag gtcacagaga tttcatcaag 300  
aacatgatta ctggtacttc tcaagctgac tgtgctatct tgattattgc tgggtggtgct 360  
gggtgaattcg aagccggtat ctctaaggat ggtcaaacca gagaacacgc tttgttggtc 420  
ttcaccttgg gtggttagaca attgattgtt gctgtcaaca agatggactc cgtcaaatgg 480  
gacgaatcca gattccaaga aattgtcaag gaaacctcca actttatcaa gaaggttggt 540  
tacaacccaa agactgttcc attcgtccca atctctgggt ggaacggtga caacatgatt 600



gaagctacca ccaacgctcc atggtacaag ggttgggaaa aggaaaccaa ggccggtgtc 660  
 gtcaagggtg agactttgtt ggaagccatt gacgccattg aacaaccatc tagaccaact 720  
 gacaagccat tgagattgcc attgcaagat gtttacaaga ttggtggtat tgggtactgtg 780  
 ccagtcggta gagttgaaac cgggtgtcatc aagccaggta tggttgttac ttttgcccca 840  
 gctggtgtta ccaactgaagt caagtccgtt gaaatgcatc acgaacaatt ggaacaagggt 900  
 gttccagggtg acaacgttgg tttcaacgtc aagaacgttt ccgttaagga aatcagaaga 960  
 ggtaacgtct gtggtgacgc taagaacgat ccaccaaagg gttgcgcttc tttcaacgct 1020  
 accgtcattg ttttgaacca tccagggtcaa atctctgctg gttactctcc agttttggat 1080  
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 tctggtaaga agttggaaga ccatccaaag ttcttgaagt ccggtgacgc tgctttgggtc 1200  
 aagttcgttc catctaagcc aatgtgtgtt gaagctttca gtgaataccc accattaggt 1260  
 agattcgctg tcagagacat gagacaaact gtcgctgtcg gtgttatcaa gtctgttgac 1320  
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<210> 493  
 <211> 2865  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 493

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 gtaagggttca ctataaatga gaaagagaga atgccaacca acagcagcgg tttgttgatc 360  
 tcttcacaac ggttcaatga gacctggaag tacgcattcg acaagaaatt tcaagaggag 420  
 gcgaacagga ccagtattcc acaattccac ttccttaagc aaaaacaaac tgtgaactca 480  
 ttctggtcga aaatatcttc atttttgtca ctttcaaact ccactgcaga cacatttcat 540  
 cttcgaaacg gtgatgtatc cgtagaaatc tttgctgaac cttttcaatt gaaagtttac 600



caattttact ggagtaattc aggtctatta gtcaaacctg tcacggagcc tggatcaatca 2280  
 gaaacggaaa tgggtttccc acccggtata ttctatgaat tcgcatcttt acactctttt 2340  
 ataaacaatg gtactgattt gatagaaaag aatatttctg caccattgga taaaattcca 2400  
 ttattttattg aaggcgggtca cattatcact atgaaagata agtatagaag atcttcaatg 2460  
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 actcagttca ttttcgaaaa caatacctta aaaaatgttc gaagtcatat tcccgagaat 2640  
 ttgacaggca ttcaccacaa tactttgagg aataccaata ttgaaaaaat cattatcgca 2700  
 aagaataatt tacaacacaa cataacgttg aaagacagta ttaaagtcaa aaaaaatggc 2760  
 gaagaaagtt cattgccgac tagatcgtca tatgagaatg ataataagat caccattctt 2820  
 aacctatcgc ttgacataac tgaagattgg gaagttattt ttgga 2865

<210> 494  
 <211> 786  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 494

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 catccacaaa tagaccaggc actaacaaaa catcgtacgt tgaatgagtc gcgtttattg 240  
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 aatggattca tttgggttaga gttccttgga gaagatcttc ccggagggca cggttttagt 360  
 aacctaaga acttcctttg gatgcatgac caagatccat atagtgatct tgtagcaact 420  
 acactacgga aagtggggcg ccaaattggg ttgttgcaact ggaatgacta ctgtcatggc 480  
 gatttgacaa gttctaacat tgttctcgtg cgagatgggt cgagatggac gcctcatttg 540  
 attgatttcg gtctgggctc agtttcaaac ctggtcgaag ataaaggcgt cgatttatac 600  
 gtcttagaga gagctatttt aagtacacat tcgaagcatg cggaaaaata caatgcttgg 660  
 atcatggagg ggttcgaaga ggtctatcgt gaacaagggt cgaaagggtc caagaaactg 720

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 ggataa 786

<210> 495  
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 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 495

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 atgcaggccc ctaaagaaga tgcaaaccgg ttgtcaaagg ggtttctccg attgaatgaa 240  
 acagcttttt tatattacat gatcgtatat acgctcttgg aggatactct accaagacta 300  
 aaggaatfff cgagcaacaa ggaccagaat gttagaaatt tgtacgggga gagaatccag 360  
 cttttgcata atgacccaaa tattgagcgg attcgaaatg tcattgaaaa ttaccctaaa 420  
 tttatacagt tgcaaaactat agagcctgga aaattaagtt caatgttgca ttttcatggt 480  
 gacgctctct tgcttatcga tgtacggccc aggtctgaat tcgttagggc ccatatcaaa 540  
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 attgaaagtg tctctttgat cacttcgccg cactcagata tcactttctt ttcaaatega 660  
 gacaagttca agttcattat tttatatact gacactcaat tgcataataa tttccagcaa 720  
 aggcaaaact gcatccttgc gaagattctt tcccaaaatt cagtaattaa accgctgagt 780  
 gggacgaaaa tactcatfff ggaaaatggg ttctcaaact gggttaaatt aggtggggcg 840  
 tatcaatcat ctgtcagtga aacagcgcat cttacatcct cgtcttcaac gccagctfff 900  
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 aagagtatgc cgatggatc catgaatacg cagcctttgc taacaacggg gcaaaggcct 1020  
 caattaccct tatactattc agatttgccc attataccgc aaccaagccc caacagaaat 1080  
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 ccctcgacca ttcaaaaata agcaaact gtggaaagaa tatcgccaga tatccgcgct 1200  
 gcacaagccc atgcctatff gccccctgca tcgaatgtat tctcgccacg tattccgcct 1260

ttaccacaac agaacttgtc ttcgtcgagg cagaccatcc taaacaactc acaagtgcctt 1320  
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 cttcaatggt tgggtgggaac acacgatttg gttcgaatgt ttttggataa cacatatctg 1440  
 aattttatta attttgatag ttcaagaggt tctaaggggt tgctagctaa aaatttcgcc 1500  
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 ccagttcaga ctattcagtt taaaaaatt tgcggccata tcaaccccat gtatagtgat 1620  
 tcaatgcagc aagattgtca agaattttgt caatttttgc ttgatggctt gcacgaggat 1680  
 ttgaaccaa acggcagcaa aaagcatctt aaacagttat cagatgagga agaacgaatg 1740  
 agagaaaaaa tgtccatacg caaagctagt gctctcgagt gggaaagatt tttgcttacc 1800  
 gacttcagtg cgataattga ccttttccaa ggacagtacg catctaggct acaatgtcaa 1860  
 gtttgtgaac atacctccac aacttaccaa acattctctg ttctttctgt tcctgtccca 1920  
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 ggtgtcgatg aacaatgggtc atgtcctaaa tgcttaaaaa agcagccttc cactaaacaa 2040  
 ctgaagatta ctagattgcc taagaaacta attattaatt tgaaacgatt tgacaatcaa 2100  
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 gcgagagatt ttaatcatga agctattggt aatgaggaca ttcctaccag gggccaagta 2220  
 ccaccattta gatacagact gtatgggggt gcatgtcatt cggggagttt gtatggggga 2280  
 cactatactt catacgttta taagggacca aaaaaagggt ggtatttttt cgatgactcg 2340  
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<210> 496  
 <211> 2295  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 496

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 acttcggttg catcattaaa ggctatccga tcaggaaatg aagaggaaag cggaacgag 180



atgaccaata cagaattcaa tgttttgatg aaagagagta agtccattgg ccccgatggg 1860  
 ttatctttca atgaaaactt caatactact ccagaaggat ttgcgccgtc gattgatccg 1920  
 ggtgaggaga gtaatgatac tgtactagca ccagtcccag gctcaacgat aaggaaacca 1980  
 cgcacttggt ttggcggttg ttacgcggtg actggtatgg atcagtgggt agcgggtata 2040  
 aaagaaacca ttggcatcaa ggatagcacc ggacataatg tgtattcgat tacatcaaga 2100  
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<210> 497  
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 caagaaaaga ttctaagagt catagtgcc aagggtcaga attgttctat ttgtctgagc 600  
 gaggaaccgg tggctcccag aatggttact tgtggccata ttttctgtct aagttgtctc 660  
 ttgaactttt tctccattga agaaaccgtc aaaaataaag agactgggta ctcaaagaag 720  
 aagaaatata aagaatgcc gctttgtgga agtattattg gccctaaaag ggtcaagcct 780  
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 <213> *Saccharomyces cerevisiae*

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<213> Saccharomyces cerevisiae

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 <212> DNA

<213> Saccharomyces cerevisiae

<400> 516

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<400> 519

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<400> 521

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<213> Saccharomyces cerevisiae

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<211> 2175

<212> DNA

<213> Saccharomyces cerevisiae

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1878

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<211> 4434

<212> DNA

<213> *Saccharomyces cerevisiae*

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<213> Saccharomyces cerevisiae

<400> 528

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<212> DNA

<213> Saccharomyces cerevisiae

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<400> 534

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<212> DNA  
<213> *Saccharomyces cerevisiae*

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 <212> DNA  
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<400> 538

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<213> *Saccharomyces cerevisiae*

<400> 540

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<213> Saccharomyces cerevisiae

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<210> 542

<211> 1836

<212> DNA

<213> Saccharomyces cerevisiae

<400> 542

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 <211> 858  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 543

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<400> 544

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2595

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 <213> *Saccharomyces cerevisiae*

<400> 547

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<213> *Saccharomyces cerevisiae*



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gcattgaagg acttaagtgt tgacgaattc aaagggttaca tacaggatcc tctcacgat 420

gagactatac cactgacgtt gccactgggt gataaaaaaa tcagcctacc cagttttata 480

acgccgccaa gaaattcgaa gatattctatt ttcttacta gtaaacaacca aggacagaac 540

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<210> 554

<211> 1671

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 554

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acgaaatcaa cagattcaaa ctgtggaact aaatgctctg gctggcctta ccaaattgtgt 360

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tcctccgcc aaatttcttc aactactagg agaacgtcaa cagatatgaa aagttctgaa 540

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 555

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<212> DNA  
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<400> 557

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<400> 563

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<210> 566  
<211> 363  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 566

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<210> 567  
<211> 1908  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 567

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 <211> 417  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

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aaaaataccg gtgaacctct ggggacaaga attatggcta atgatgggtg tgtagataga 360  
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<210> 569

<211> 768

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 569

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gtaaaaaata gcgaactacc tagtgtgctg gttaatgaaa tggtcggaac caaagtaact 720  
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<210> 570

<211> 324

<212> DNA

<213> Saccharomyces cerevisiae

<400> 570

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gatgctaaac gagtcgaaga aaagaggttg agaaagacaa acggagttgc atcattagat 180  
cccacaaaag aaaggaaaag gtacttcaat atgagtgaac acgaggaaca aaaagagttg 240  
cgaaagaagt atgagaccat gcaaccgctt agtggagaag ttgtgaccaa agatggagag 300  
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<210> 571

<211> 936

<212> DNA

<213> Saccharomyces cerevisiae

<400> 571

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936

<210> 572  
<211> 3294  
<212> DNA  
<213> *Saccharomyces cerevisiae*

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<210> 573  
 <211> 1086  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <400> 573

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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 574

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<210> 575  
<211> 489  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 575

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 agtaaatga 489

<210> 576  
 <211> 1473  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 576

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 caagaaattg tggcaattaa ggtggtcaac ctggagcatt ccgacgaaga cattgaactg 180  
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<211> 2988  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 577

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gctaacgact ccgatagtga agttgaagct gattatggtc ccaacgatgt ccaggatgtg 180  
atcgagtaca gtcagatga agaagaagga gtgaacaata agaagaaggc tgaaaacaag 240  
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tcagatgatg aaaacaatgc atctggtaaa actcaaaccg gtgatgatga ggatgatgtc 360  
aatgaatatt tctccacaaa taacttggag aaaacaaagc ataaaaaagg tagttttccc 420  
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 aagaaggtgg aaaaggtctc acaaagtgg aatttccgtca agggatataa taacgctccg 2880  
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<210> 578  
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 <212> DNA  
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 <400> 578

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 acggcggaaa attcggattt gaaggaaaa atgaactgca agaatacgct caatgagtac 240  
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aacacgcaaa tcaacaaact tcgcatcgt atagagcaat tgaacaagga aaatgagttt  480
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<212>      DNA
<213>      Saccharomyces cerevisiae

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<213>      Saccharomyces cerevisiae

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aagttcaaca agaacaagaa tatcaacact aatgtgtatg tggacaactc ctcaatagag  180

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tatgttgagg acttcgagca agattggaag aatgagttac gtagggatga cctctgcgat 1860  
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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 582

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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 583

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<211> 1152  
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<400> 584

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<400> 585

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<213> *Saccharomyces cerevisiae*

<400> 587

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<212> DNA  
<213> *Saccharomyces cerevisiae*  
<400> 588

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<400> 590

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 <213> *Saccharomyces cerevisiae*

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 <213> *Saccharomyces cerevisiae*

<400> 592

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<212> DNA  
<213> *Saccharomyces cerevisiae*

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<210> 594  
<211> 387  
<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 594  
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<400> 595

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603



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 agggaaggcc aagctgttgt tcaatggatg cggttcgagg ccacagcgaa ggggggcgct 300  
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 <212> DNA  
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<400> 607

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<400> 610

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1395

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<400> 614

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 <212> DNA  
 <213> *Saccharomyces cerevisiae*

<400> 615

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<212> DNA  
<213> *Saccharomyces cerevisiae*

<400> 618

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<211> 462  
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<213> *Saccharomyces cerevisiae*

<400> 619

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<210> 620  
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<213>      *Saccharomyces cerevisiae*

<400>      620

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1929

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<210>      622
<211>      358
<212>      PRT
<213>      Glycine max
<400>      622

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35        40        45
Lys Pro Phe Asn Pro Ile Leu Gly Glu Thr Tyr Glu Met Val Asn His
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Ser Ala Gly His Ala Glu Thr Glu His Phe Thr Tyr Asp Val Thr Ser

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 <211> 300  
 <212> PRT  
 <213> Glycine max

<400> 623

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65					70					75					80
Pro	Asp	Pro	Lys	Phe	Asn	Gly	Thr	Ser	Val	Glu	Ala	Lys	Val	His	Gly
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Ala	Gly	Thr	Val	Asn	Ile	Arg	Cys	Leu	Glu	Thr	Gly	Leu	Val	Ala	Glu
		130				135					140				
Leu	Ser	Tyr	Arg	Ser	Ser	Ser	Phe	Leu	Gly	Ile	Gly	Gly	Asn	His	Arg
145					150					155					160
Val	Ile	Lys	Gly	Lys	Ile	Leu	Asp	Ser	Ser	Ser	Leu	Lys	Val	Leu	Tyr
			165					170						175	
Glu	Val	Asp	Gly	His	Trp	Asp	Arg	Thr	Val	Lys	Val	Lys	Asp	Thr	Asn
		180						185					190		
Asn	Gly	Lys	Val	Arg	Val	Ile	Tyr	Asp	Ala	Lys	Glu	Val	Met	Ser	Gly
		195					200					205			
Leu	Glu	Thr	Pro	Ile	Leu	Lys	Asp	Ile	Glu	Gly	Val	Trp	Gln	Thr	Glu
		210				215					220				
Ser	Ala	His	Val	Trp	Gly	Glu	Leu	Asn	Gln	Ala	Ile	Val	Ser	Lys	Asp
225					230					235					240
Trp	Glu	Lys	Ala	Arg	Glu	Ala	Lys	Leu	Lys	Val	Glu	Glu	Arg	Gln	Arg
			245						250					255	

Glu Leu Val Arg Glu Arg Glu Ser Lys Gly Glu Thr Trp Ile Ser Lys  
260 265 270

His Phe Val Val Ser Asn Asn Lys Glu Gly Trp Gln Cys Ser Pro Ile  
275 280 285

His Lys Ser Val Pro Ala Ala Pro Ile Thr Ala Leu  
290 295 300

<210> 624  
<211> 355  
<212> PRT  
<213> Glycine max

<400> 624

Met Ala Glu Leu Met Glu Tyr Ser Tyr Leu Leu Asp Met Ala Asp Lys  
1 5 10 15

Thr Glu Asp Pro Tyr Met Arg Leu Val Tyr Ala Ser Ser Phe Phe Ile  
20 25 30

Ser Val Tyr Tyr Ala Tyr Gln Arg Thr Trp Lys Pro Phe Asn Pro Ile  
35 40 45

Leu Gly Glu Thr Tyr Glu Met Val Asn His Gly Gly Ile Thr Phe Ile  
50 55 60

Ser Glu Gln Val Ser His His Pro Pro Met Ser Ala Gly His Ala Glu  
65 70 75 80

Thr Glu His Phe Thr Tyr Asp Val Thr Ser Lys Leu Lys Thr Lys Phe  
85 90 95

Leu Gly Asn Ser Val Asp Val Tyr Pro Val Gly Arg Thr Arg Val Thr  
100 105 110

Leu Lys Arg Asp Gly Val Val Leu Asp Leu Val Pro Pro Pro Thr Lys  
115 120 125

Val Ser Asn Leu Ile Phe Gly Arg Thr Trp Ile Asp Ser Pro Gly Glu  
130 135 140

Met Ile Leu Thr Asn Leu Thr Thr Gly Asp Lys Val Val Leu Tyr Phe  
145 150 155 160

Gln Pro Cys Gly Trp Phe Gly Ala Gly Arg Tyr Glu Val Asp Gly Tyr  
165 170 175

Val Tyr Asn Ser Ala Asp Glu Pro Lys Ile Leu Met Thr Gly Lys Trp  
180 185 190

Asn Glu Ala Met Asn Tyr Gln Val Cys Asp Ser Glu Gly Glu Pro Leu  
195 200 205

Pro Gly Thr Glu Leu Lys Glu Ile Trp Arg Val Ala Asp Thr Pro Lys  
210 215 220

Lys Asp Lys Phe Gln Tyr Thr His Phe Ala His Lys Ile Asn Ser Phe  
225 230 235 240

Asp Thr Ala Pro Lys Lys Leu Leu Ala Ser Asp Ser Arg Leu Arg Pro  
245 250 255

Asp Arg Met Ala Leu Glu Lys Gly Asp Leu Ser Thr Ser Gly Tyr Glu  
260 265 270

Lys Ser Ser Leu Glu Glu Arg Gln Arg Ala Glu Lys Arg Asn Arg Glu  
275 280 285

Ala Lys Gly His Lys Phe Thr Pro Arg Trp Phe Asp Leu Thr Asp Glu  
290 295 300

Val Thr Pro Thr Pro Trp Gly Asp Leu Glu Val Tyr Gln Tyr Asn Gly  
305 310 315 320

Lys Tyr Thr Gln His Cys Ala Ala Val Asp Ser Ser Glu Cys Ile Glu  
325 330 335

Val Pro Asp Ile Arg Pro Glu Phe Asn Pro Trp Gln Tyr Asp Asn Leu  
340 345 350

Asp Ala Glu  
355

<210> 625

<211> 414

<212> PRT

<213> Zea mays

<400> 625

Met Ala Thr Lys Glu Glu Ala Ser Ala Val Pro Ala Ala Ser Lys Thr  
1 5 10 15

Ser Trp Ser Ser Phe Leu Lys Ser Ile Ala Ser Phe Asn Gly Asp Leu  
20 25 30

Ser Ser Leu Thr Ala Pro Pro Phe Ile Leu Ser Thr Thr Ser Leu Thr  
35 40 45

Glu Tyr Ser Ala Tyr Trp Cys Glu His Pro Ala Leu Phe Val Ala Pro  
50 55 60

Ala Arg Glu Pro Asp Pro Ala Lys Arg Ala Leu Leu Val Leu Lys Trp

65					70					75					80
Phe	Leu	Ser	Thr	Leu	His	Gln	Gln	Tyr	Cys	Ser	Arg	Ser	Glu	Lys	Leu
				85					90					95	
Gly	Ser	Glu	Lys	Lys	Pro	Leu	Asn	Pro	Phe	Leu	Gly	Glu	Leu	Phe	Leu
			100					105					110		
Gly	Lys	Trp	Ile	Glu	Asp	Glu	Asp	Val	Gly	Glu	Thr	Arg	Leu	Ile	Ser
		115					120					125			
Glu	Gln	Val	Ser	His	His	Pro	Pro	Ala	Thr	Ala	Tyr	Ser	Ile	Val	Asn
		130				135					140				
Glu	Lys	His	Gly	Val	Glu	Leu	Gln	Gly	Tyr	Asn	Ala	Gln	Lys	Ala	Ser
145					150					155					160
Phe	Ser	Ser	Thr	Ile	Gln	Val	Lys	Gln	Leu	Gly	His	Ala	Tyr	Leu	Ser
				165					170					175	
Leu	Thr	Pro	Pro	Gly	Lys	Asp	Ala	Asn	Asn	Glu	Asp	Asp	Arg	Glu	His
			180					185					190		
Tyr	Leu	Ile	Thr	Leu	Pro	Asn	Leu	His	Ile	Glu	Ser	Leu	Ile	Tyr	Gly
		195					200					205			
Thr	Pro	Phe	Val	Glu	Leu	Glu	Lys	Ser	Cys	Lys	Ile	Ala	Ser	Ser	Thr
		210				215					220				
Gly	Tyr	Ile	Ser	Lys	Ile	Asp	Phe	Ser	Gly	Lys	Gly	Trp	Leu	Ser	Gly
225				230						235					240
Lys	Lys	Asn	Thr	Phe	Ser	Ala	Val	Leu	Tyr	Lys	Glu	Ser	Asp	Gly	Glu
				245					250					255	
Lys	Asn	Pro	Leu	Tyr	Thr	Ala	Asp	Gly	Gln	Trp	Ser	Ser	Ser	Phe	Thr
			260					265						270	
Ile	Arg	Asp	Ala	Arg	Ala	Lys	Lys	Asp	Ile	Glu	Thr	Phe	Thr	Ile	Ser
		275					280					285			
Asn	Leu	Lys	Thr	Thr	Pro	Leu	Thr	Val	Ala	Pro	Leu	Asp	Glu	Gln	Asp
		290				295					300				
Glu	Trp	Glu	Thr	Arg	Arg	Ala	Trp	Arg	Asp	Val	Ala	Ala	Ala	Ile	Glu
305					310					315					320
Arg	Gly	Asp	Met	Glu	Ala	Thr	Ser	Asn	Ala	Lys	Thr	Lys	Ile	Glu	Val
			325						330					335	
Ala	Gln	Arg	Glu	Leu	Arg	Lys	Lys	Glu	Lys	Glu	Gln	Gly	Glu	Glu	Trp
			340					345					350		
Glu	Arg	Arg	Phe	Phe	Lys	Arg	Val	Asn	Glu	Lys	Asp	Glu	Pro	Thr	Phe



355

360

365

Met Arg Leu Ala Ala Met Leu Asp Leu Thr Gln Gly Ile Glu Ser Asp  
 370 375 380

Arg Thr Gly Gly Val Trp Arg Phe Asp Pro Ser Arg Ala Val Asp Ala  
 385 390 395 400

Asn Pro Pro Tyr His Lys Val Gly Gly Glu Gly Leu Gly Leu  
 405 410

<210> 626

<211> 434

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 626

Met Ser Gln His Ala Ser Ser Ser Ser Trp Thr Ser Phe Leu Lys Ser  
 1 5 10 15

Ile Ser Ser Phe Asn Gly Asp Leu Ser Ser Leu Ser Ala Pro Pro Phe  
 20 25 30

Ile Leu Ser Pro Thr Ser Leu Thr Glu Phe Ser Gln Tyr Trp Ala Glu  
 35 40 45

His Pro Ala Leu Phe Leu Glu Pro Ser Leu Ile Asp Gly Glu Asn Tyr  
 50 55 60

Lys Asp His Cys Pro Phe Asp Pro Asn Val Glu Ser Lys Glu Val Ala  
 65 70 75 80

Gln Met Leu Ala Val Val Arg Trp Phe Ile Ser Thr Leu Arg Ser Gln  
 85 90 95

Tyr Cys Ser Arg Ser Glu Ser Met Gly Ser Glu Lys Lys Pro Leu Asn  
 100 105 110

Pro Phe Leu Gly Glu Val Phe Val Gly Lys Trp Lys Asn Asp Glu His  
 115 120 125

Pro Glu Phe Gly Glu Thr Val Leu Leu Ser Glu Gln Val Ser His His  
 130 135 140

Pro Pro Met Thr Ala Phe Ser Ile Phe Asn Glu Lys Asn Asp Val Ser  
 145 150 155 160

Val Gln Gly Tyr Asn Gln Ile Lys Thr Gly Phe Thr Lys Thr Leu Thr  
 165 170 175

Leu Thr Val Lys Pro Tyr Gly His Val Ile Leu Lys Ile Lys Asp Glu  
 180 185 190

